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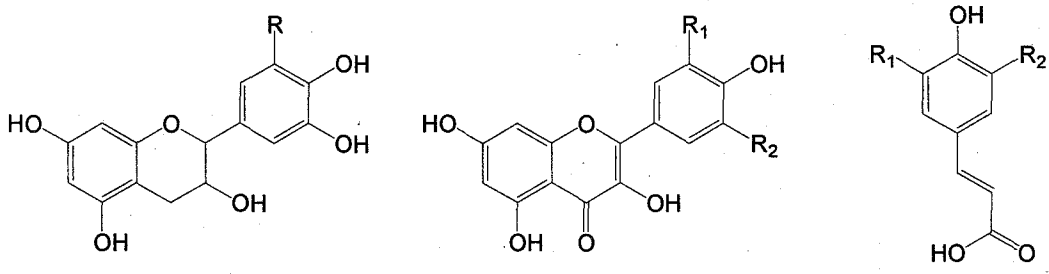
CONTENTS

General Introduction

Polyphenols, which are in a higher class of plants, are normally regarded as a group of compounds containing multiple phenolic functionalities.[1] These compounds possess important biological activities. They are expected to be in use as drugs for heart ailments, mutagenesis, etc. However, naturally occurring polyphenols have limitations due to the limited number of structural variety and complexity of the building blocks might make analysis difficult. For example, several types of polyphenols are well known. The classification of naturally occurring polyphenols is based on repeated building blocks as shown in Chart 1.[2]

Chart 1.

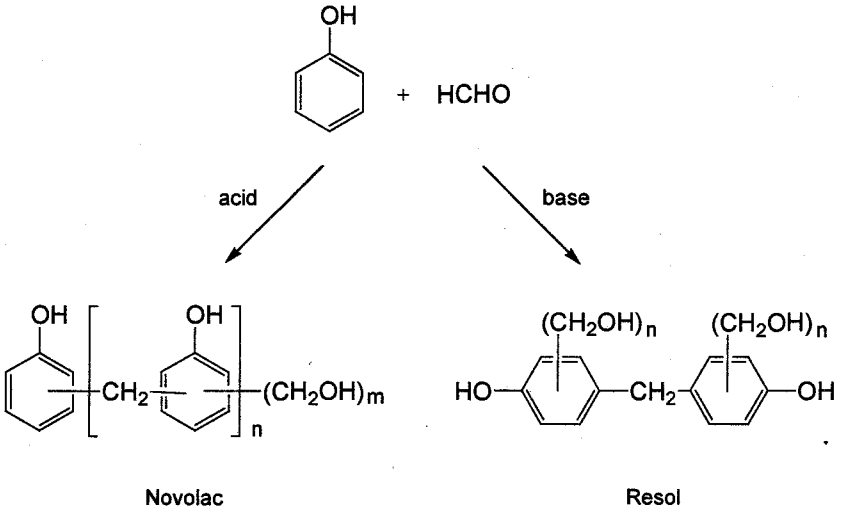


The most abundant polyphenol is lignin, which can be seen in woody tissues, while it is often excluded from the category of so-called polyphenols. Lignin also has extremely complicated structure, and kinds of the repeating units are numerous. It is noted that lignin is biologically degraded in spite of the low solubility and the variety of the structure.[3] Interestingly, it is reported that lignin is formed by a random polymerization of *p*-coumaryl alcohol radicals and its methoxy-substituted derivatives in vitro.[4]

On the other hand, synthetic polyphenols usually have simpler repeating unit compared to natural ones. Phenol-formaldehyde resins (Scheme 1) would be the most conventional synthetic polyphenols. Novolacs and resols are widely used in industrial

fields because of the excellent toughness and thermal properties.[5] Novolacs are prepared by reacting phenol with formaldehyde under acidic conditions, in which the molar ratio affects the degree of crosslinking. On the other hand, resols are prepared under alkaline conditions. Thus the structure of these phenol-formaldehyde resins is highly dependent on the reaction conditions and various kinds of related compounds have been in use. However, the toxicity of formaldehyde causes problems in the practical use. It is strongly demanded to develop an alternative way for preparation of polyphenols.

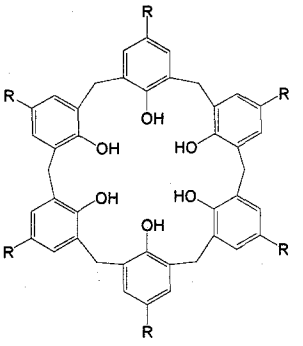
Scheme 1.



Novolac Resol

p-Alkylphenols give cyclic oligomers upon condensation with aldehydes under specific reaction conditions, which are called calixarenes and also regarded as polyphenols.[6,7] The ring size varies depending on reaction conditions.[7] Tetra-, hexa-, and octameric products are synthesized in basic condition, whereas acid catalysis afforded a special calixarene. The unique conformations have been paid much attention. Calixarenes provide aromatic cavity, which can make complexes with metal ions bearing suitable size,[8] depending on the conformation.

Chart 2.

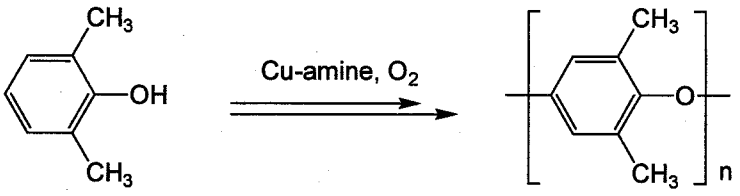


Calix[6]arene

Another representative polyphenol is

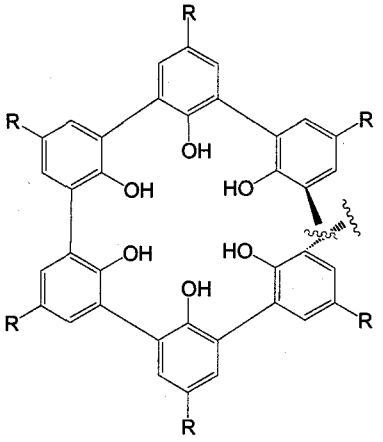
poly(phenylene oxide) (PPO), which is prepared by copper/amine catalyst from 2,6-dimethylphenol (Scheme 2).[9,10] PPO shows good mechanical properties and chemical stability even at high temperatures and is used as engineering plastics.[11] The essential difference of PPO from other polyphenols is that PPO has only one phenolic hydroxyl group in a single polymer chain and consists of C-O linkage. This is the characteristic point of PPO and brings about unique properties.

Scheme 2.

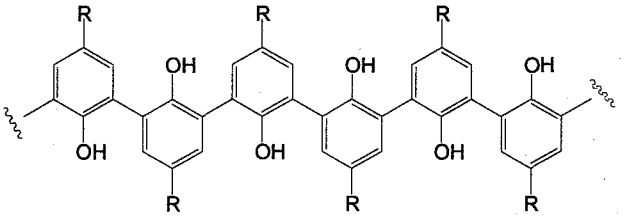


Unlike PPO, synthesis of a polyphenol consisting of only C-C linkage is reported, that is an *ortho*-phenol polymer.[12,13] The structure potentially has two conformational isomers, *cisoid* and *transoid* (Chart 3). These two isomers are expected to differ in complexation ability due to the orientation of multifunctional hydroxyl groups.[14]

Chart 3.



Cisoid



Transoid

Thus extensive work on polyphenols has been reported and some of them have been practically applied. It is a quite significant work to develop novel polyphenol synthesis. Utilization of enzymatic polymerization to polyphenol synthesis has been greatly paid attention[15-32] as one way not only to solve some environmental problems concerned with formaldehyde but also to gain a novel method for preparation of new functional polyphenols.

Horseradish peroxidase C (HRPC), which is one of the well studied enzymes as the model for Michaelis-Menten kinetics, is a kind of heme glycoproteins having a 42 kDa single chain of 308 residues with eight oligosaccharide side chains, two calcium ions, and one prosthetic protoporphyrin IX.[33-38] The normal catalytic cycle is shown in Scheme 3, where compound I, compound II, and AH_2 represent a two-electron oxidized form of the enzyme, a one-electron oxidized form of the enzyme, and electron donor, respectively. The essential catalytic residues for the activity are His42 and Arg38 in the distal site. His42 serves as an acid-base catalyst,[39] whereas Arg38 makes enzyme more active by facilitating charge separation. Asn70 and Glu64 are also important to maintain the activity by forming the network His42-Asn70-Glu64 via hydrogen bonds.[40,41] The active site structure of HRP obtained by crystallographic study[42] is shown in Figure 1.

Scheme 3.

$$\text{HRP (resting state)} + H_2O_2 \longrightarrow \text{HRP (compound I)} + H_2O$$

$$\text{HRP (compound I)} + AH_2 \longrightarrow \text{HRP (compound II)} + AH^{\cdot}$$

$$\text{HRP (compound II)} + AH_2 \longrightarrow \text{HRP (resting state)} + AH^{\cdot}$$

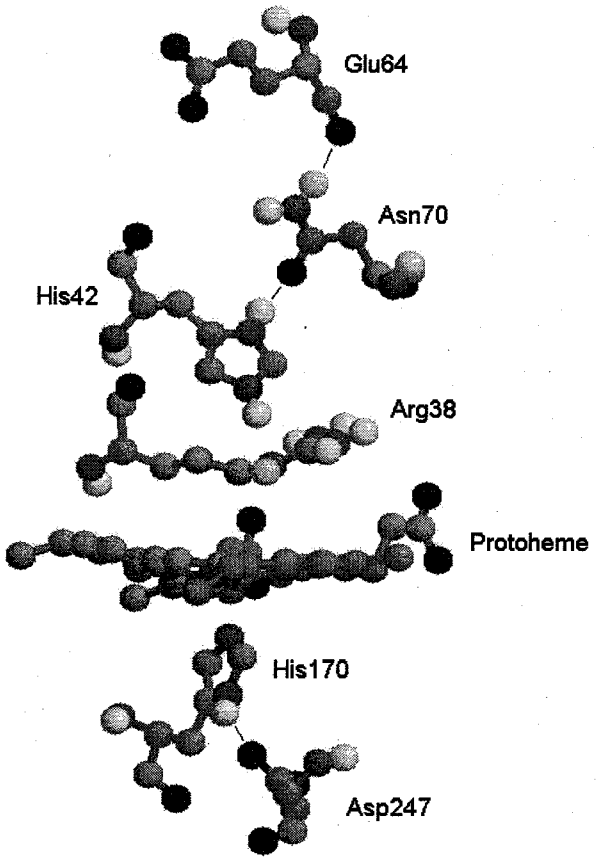
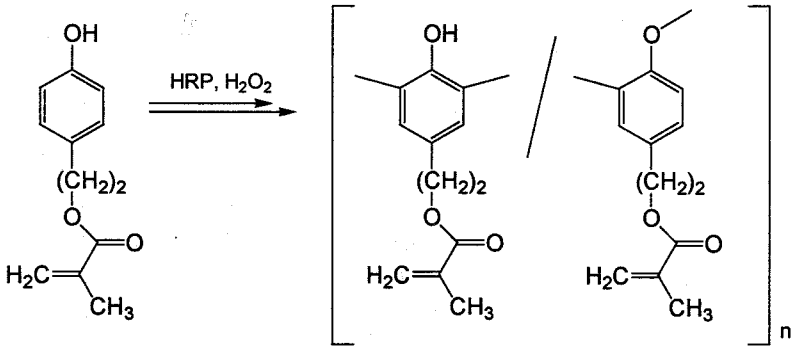


Figure 1. Active site structure of HRP. The hydrogen bonds are indicated by solid lines.

HRP catalytically oxidizes a wide variety of aromatic substrates to free radicals, typically phenols to phenoxy radicals, at the expense of hydrogen peroxide.[43] In case of the phenolic monomers having unsubstituted *o*- and/or *p*-positions, the resulting phenoxy radicals easily couple with each other step by step to give higher molecular weight products. Since HRP has been reported to maintain its activity even in a mixture of buffer and water-miscible organic solvents, HRP-catalyzed polymerizations of aromatic compounds have been extensively studied in such media. This process does not use toxic formaldehyde and their synthetic procedure is very facile. Some of the polyphenols showed high thermal stability. In the peroxidase-catalyzed polymerization of a phenol derivative having methacryloyl group, the phenolic moiety was

chemoselectively polymerized to give a polymer having the methacryloyl group in the side chain (Scheme 3).[29] The resulting polymer had a structure consisting of phenylene and oxyphenylene units. The polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid) involved the elimination of hydrogen and carbon dioxide from the monomer to give PPO having a carboxylic acid group at one end and a phenolic group at the other.[44] Interestingly, conventional chemical oxidation catalysts did not induce the polymerization of syringic acid.

Scheme 4.



Considering polyphenols as a chemical substance, it is noted that polyphenols can be oxidized and simultaneously prevent free radical formation. Such a reaction is considered to be the essential step for acting as drugs. The other important aspect is binding with biologically important molecules by the multiple functional groups. For example, polyphenols strongly interact with proteins. This phenomenon is also considered to be a significant property for the use in medical fields. Chemical modification and novel synthetic pathway would improve the properties and give more potential to polyphenols.

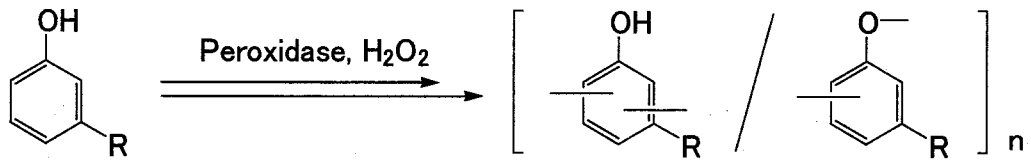
With the background described above, the present thesis consists of seven chapters including the following topics on the oxidative polymerization of phenolic compounds: preparation of new functional polyphenols, establishment of efficient

reaction conditions, and development of novel reaction systems.

A comprehensive classification of polyphenols including natural and synthetic one has not been complete. The definition of the word 'polyphenol' is not clear and sometimes confusing. Therefore 'phenolic polymers' is used below to represent polymers obtained by polymerization of phenolic monomers.

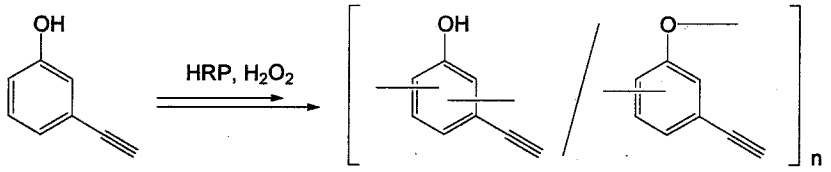
In Chapter 1, HRP- and soybean peroxidase (SBP)-catalyzed oxidative polymerization of *m*-substituted phenols has been performed in a mixture of a water-miscible organic solvent and buffer at room temperature under air (Scheme 5). In the polymerization of *m*-cresol using HRP catalyst, effects of an organic solvent, buffer pH, and their mixing ratio have been systematically investigated with respect to the polymer yield, solubility, and molecular weight. The difference of the polymerization behaviors, depending on the origin of peroxidases, and relationship with the *m*-substituent were examined.

Scheme 5.



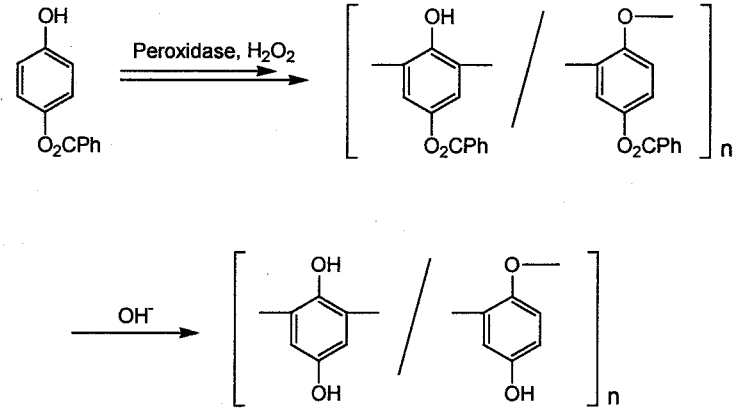
In Chapter 2, HRP-catalyzed polymerization of *m*-ethynylphenol possessing two reactive groups, phenol and acetylene moieties, was carried out in an aqueous methanol under air (Scheme 6). The reaction of the monomer using a copper/amine catalyst, a conventional catalyst for oxidative coupling, exclusively produced a diacetylene derivative. On the other hand, the peroxidase catalysis induced the chemoselective polymerization of the monomer. The resulting polymer was converted to carbonized polymer in a high yield and the process was analyzed by IR, Raman, and X-ray diffraction measurements.

Scheme 6.

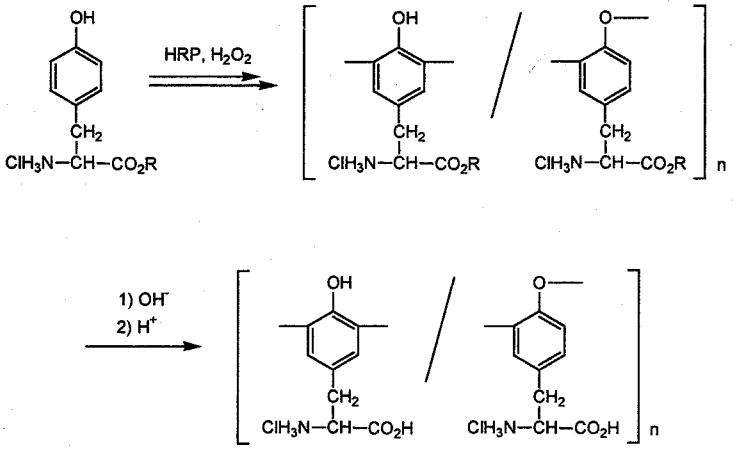


In Chapter 3, two functional phenolic polymers were chemoenzymatically prepared. At first, 4-hydroxyphenyl benzoate was oxidatively polymerized by the peroxidase catalyst and followed by hydrolysis in alkaline solution to give poly(hydroquinone) (Scheme 7). The polymerization of tyrosine esters, followed by alkaline hydrolysis of the ester group, produced the other target, poly(tyrosine) having amino acid moiety in the side chain (Scheme 8).

Scheme 7.

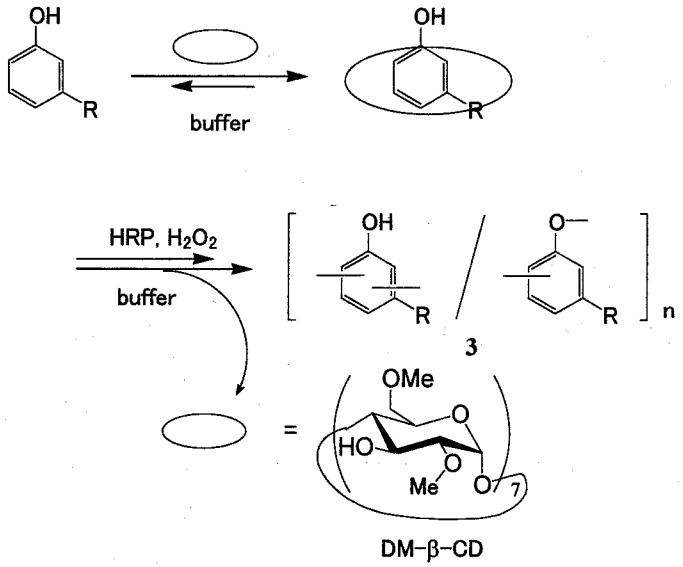


Scheme 8.



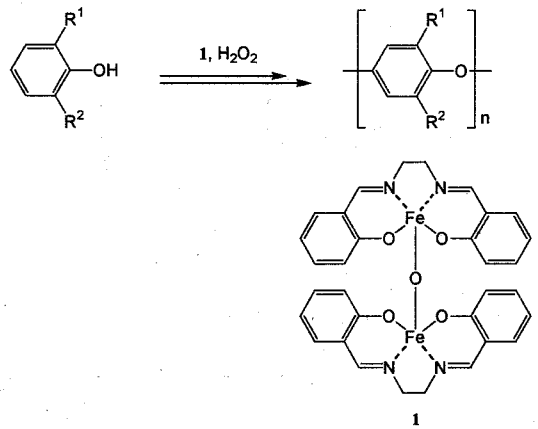
In Chapter 4, HRP-catalyzed polymerization of *m*-substituted phenols has been achieved in the presence of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) in a buffer (Scheme 9). A water-soluble complex of the monomer and DM-β-CD was formed and the polymerization was performed by peroxidase catalyst to give the polymer in high yields. The inclusion complex formation was examined by NMR.

Scheme 9.



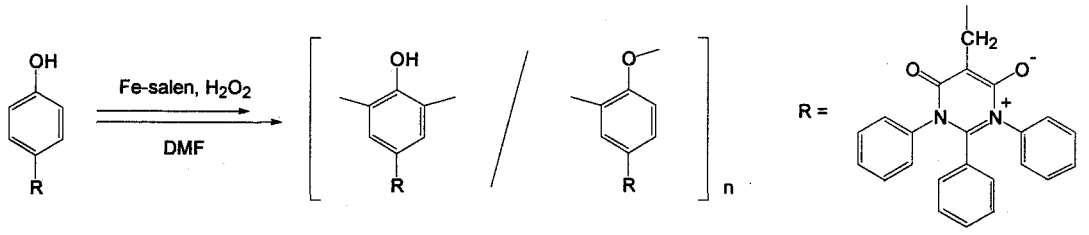
In Chapter 5, oxidative polymerization of 2,6-disubstituted phenols has been performed by using an iron salen complex and hydrogen peroxide as a catalyst and an oxidizing agent, respectively (Scheme 10). Efficient production of PPO and potential of the complex for oxidative polymerization were mentioned.

Scheme 10.



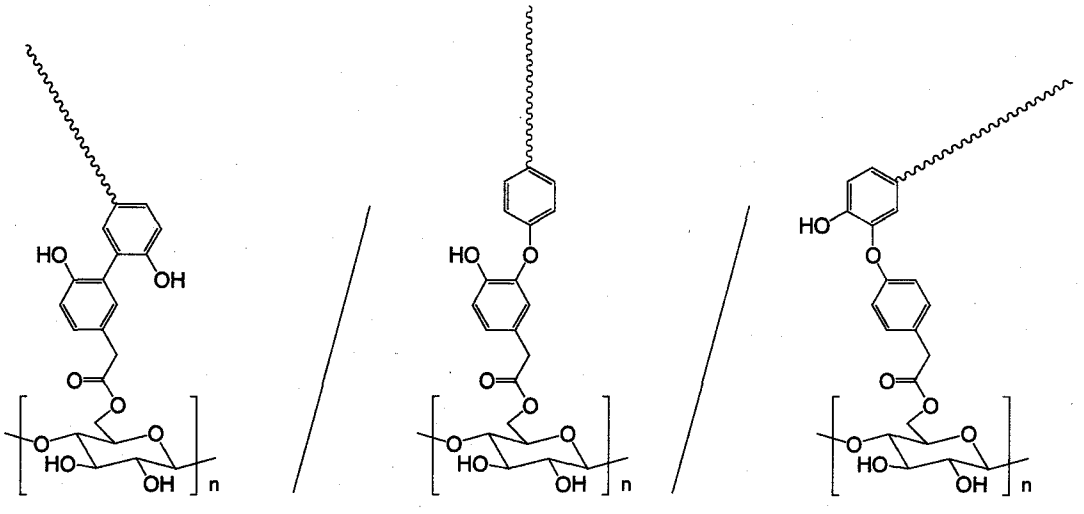
In Chapter 6, synthesis of a phenolic polymer bearing photosensitive groups was carried out (Scheme 11) and its photochemical behavior caused by UV irradiation was discussed. Spin coated polymer films were prepared and characterized. The behavior in the UV irradiation was analyzed by IR spectroscopy and waveguide spectroscopy.

Scheme 11.



In Chapter 7, oxidative grafting of phenolic polymers onto a phenol-containing cellulose has been performed in homogeneous system at room temperature under air to produce cellulose-phenolic polymer hybrids (Chart 4). The course of the reaction was analyzed by SEC in detail.

Chart 4.



The present investigation provides not only new functional phenolic polymers but also environmentally benign and efficient reaction systems to produce soluble

polymers by utilizing new catalyst and method, which would be further applicable to synthesis of a variety of functional phenolic polymers for the future. The experimental results will be described in the following chapters.

References

1) Tuckmantel, W.; Kozikowski, A. P.; Romanczyk, L. J., Jr.; *J. Am. Chem. Soc.* **1999**, *121*, 12073.

2) Haslam, E. In *Practical Polyphenolics, From Structure to Molecular Recognition and Physiological Action*; Cambridge University Press: Cambridge, 1998.

3) Have, R. T.; Teunissen, P. J. M. *Chem. Rev.* **2001**, *101*, 3397.

4) Dean, J. F. D.; Eriksson, K. E. *Holzforschung* **1992**, *46*, 135.

5) Kopf, P. W. In *Encyclopedia of Polymer Science and Engineering*, 2nd ed.; John Wiley & Sons: New York, 1986; Vol. 11, p 45.

6) Knop, A.; Bohmer, V.; Pilato, L. A. In *Comprehensive Polymer Science*; Allen, G. Ed.; Pergamon Press: Oxford, 1989; Vol. 5, p 611.

7) Pochini, A.; Ungaro, R. In *Comprehensive Supramolecular Chemistry*; Atwood, J. L.; Davies, J. E. D.; MacNicol, D. D. Eds.; Pergamon: Oxford, 1996; Vol. 2, p 103.

8) Collet, A. *Tetrahedron* **1993**, *43*, 5725.

9) Hay, A. S. *J. Polym. Sci.*, **1962**, *58*, 581.

10) Hay, A. S. *J. Polym. Sci., Polym. Chem. Ed.* **1998**, *36*, 505.

11) Aycock, D.; Abolins, V.; White, D. M. In *Encyclopedia of Polymer Science and Engineering*; 2nd ed.; John Wiley & Sons: New York, 1986; Vol. 11, p 45.

12) Kobacic, P.; Jones, M. B. *Chem. Rev.* **1987**, *87*, 357.

13) Reddinger, J. L.; Reynolds, J. R. *Adv. Polym. Sci.* **1999**, *145*, 57.

14) Xu, M. H.; Lin, Z. M.; Pu, L. *Tetrahedron Letters* **2001**, *42*, 6235.

15) Oguchi, T.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1999**, *20*, 401.

16) Oguchi, T.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1389.

17) Kobayashi, S.; Shoda, S.; Uyama, H. *Adv. Polym. Sci.* **1995**, *121*, 1.

18) Kobayashi, S.; Shoda, S.; Uyama, H. In *The Polymeric Materials Encyclopedia*; Salamone, J. C. Ed.; CRC Press: Boca Raton, 1996; p 2102.

19) Kobayashi, S.; Shoda, S.; Uyama, H. In *Catalysis in Precision Polymerization;* Kobayashi, S. Ed.; John Wiley & Sons: Chichester, 1997, Chapt. 8.

20) Ritter, H. In *Desk Reference of Functional Polymers, Syntheses and Applications*; Arshady, R. Ed.; American Chemical Society: Washington, 1997; p 103.

21) Gross, R. A.; Kaplan, D. L.; Swift, G. Eds. *ACS Symp. Ser.* **1998**, *684*.

22) Kobayashi, S. *J. Polym. Sci., Polym. Chem. Ed.* **1999**, *37*, 3041.

23) Kobayashi, S.; Uyama, H.; Ohmae, M. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 613.

24) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793.

25) Liu, W.; Cholli, A. L.; Kumar, J.; Tripathy, S.; Samuelson, L. *Macromolecules* **2001**, *34*, 3522.

26) Reihmann, M. H.; Ritter, H. *Macromol. Chem. Phys.* **2000**, *201*, 798.

27) Fukuoka, T.; Tonami, H.; Maruichi, N.; Uyama, H.; Kobayashi, S.; Higashimura, H. *Macromolules* **2000**, *33*, 9152.

28) Liu, W.; Cholli, A. L.; Kumar, J.; Tripathy, S.; Samuelson, L. *Macromolecules* **2001**, *34*, 3522.

29) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.

30) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*, 31-36.

31) Rao, A. M.; John, V. T.; Gonzalez, R. D.; Akkara, J. A.; Kaplan, D. L. *Biotechnol. Bioeng.* **1993**, *41*, 531-540.

32) Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, 423-426.

33) Ortiz de Montellano, P. R. *Annu. Rev. Pharmacol. Toxicol.* **1992**, *32*, 89.

34) Poulos, T. L.; Fenna, R. E. In *metal ions in Biological Systems*; Sigel, H., Ed.; Marcel Dekker: New York, 1994; p 25.

35) Welinder, K. G. *Eur. J. Biochem.* **1979**, *96*, 483.

36) Haschke, R. H.; Friedhoff, J. M. *Biochem. Biophys. Res. Commun.* **1978**, *80*, 1039.

37) Ogawa, S.; Shiro, Y.; Morisima, I. *Biochem. Biophys. Res. Commun.* **1979**, *90*, 674.

38) Morishima, I; Kurono, M.; Shiro, Y. *J. Biol. Chem.* **1986**, *261*, 9391.

39) Poulos, T. L.; Kraut, J. *J. Biol. Chem.* **1980**, *255*, 8199.

40) Nagano, S.; Tanaka, M.; Ishimori, K.; Watanabe, Y.; Morishima, I. *Biochemistry* **1996**, *35*, 14251.

41) Tanaka, M.; Ishimori, K.; Mukai, M.; Kitagawa, T.; Morishima, I. *Biochemistry* **1997**, *36*, 9889.

42) Gajhede, M. Schuller, D. J.; Henriksen, A.; Smith, A. T.; Poulos, T. L. *Nat. Struct. Biol.* **1997**, *12*, 1032.

43) Dunfold, H. B. In *Peroxidases in Chemistry and Biology*; Everse, J. E.; Everse, K. E.; Grisham, M. B. Eds.; CRC press: Boca Raton, 1991; Vol. II, p 1.

44) Ikeda, R.; Sugihara, J.; Uyama, H.; Kobayashi, S. *Polym. International* **1998**, *47*, 295.

Chapter 1

Peroxidase-Catalyzed Oxidative Polymerization of *m*-Substituted Phenol Derivatives

Introduction

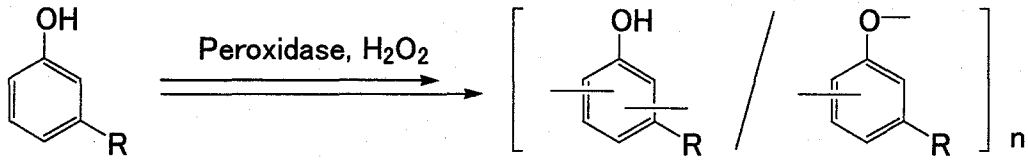
Peroxidases induced the oxidative polymerization of phenol derivatives under mild reaction conditions to produce a new class of phenolic polymers in good yields.[1-13] This process does not use toxic formaldehyde and their synthetic procedure is very facile. Some of the phenolic polymers showed high thermal stability.[7,8] In the peroxidase-catalyzed polymerization of a phenol derivative having methacryloyl group, the phenolic moiety was chemoselectively polymerized to give a polymer having the methacryloyl group in the side chain.[10] The polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid) involved the elimination of hydrogen and carbon dioxide from the monomer to give poly(1,4-oxyphenylene) having a carboxylic acid group at one end and a phenolic group at the other.[13] Interestingly, conventional chemical oxidation catalysts did not induce the polymerization of syringic acid.

A mixture of a water-miscible organic solvent and buffer was often used as a solvent of the enzymatic polymerization of phenols, in which peroxidase as well as monomer is soluble. The previous studies on the oxidative polymerization of phenols using horseradish peroxidase (HRP) as a catalyst[1-3] showed that an aqueous 1,4-dioxane system afforded the polymers in high yields. However, the resulting polymers showed low solubility toward organic solvents. Afterwards, the solubility was improved by changing the solvent composition, e.g., the polymerization of bisphenol A catalyzed by

soybean peroxidase (SBP) in an equivolume mixture of methanol and phosphate buffer (pH 7) produced the polymer, readily soluble in methanol, acetone, *N,N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO).[12]

So far, the detailed polymerization behaviors of the enzymatic polymerization of unsubstituted and *p*-substituted phenols have been investigated, whereas there have been few studies on the enzymatic polymerization of *m*-substituted monomers. Poly(*m*-cresol) was synthesized by the HRP-catalyzed polymerization in an aqueous organic solvent and a reversed micellar system,[5,6,11] and HRP did not catalyze the oxidative polymerization of *m-iso*-propylphenol in the aqueous 1,4-dioxane.[5] The present chapter describes systematical investigation on the enzymatic oxidative polymerization of *m*-substituted phenols (Scheme 1). In this study, relationships between the enzyme type and monomer substituent in the peroxidase-catalyzed polymerizations have been clearly shown for the first time.

Scheme 1.



Experimental section

Materials. *m*-Substituted phenols were commercially available and used as received. HRP (EC 1.11.1.7, 100U/mg) and SBP (EC 1.11.1.7, 60U/mg) were purchased from Wako Pure Chemical Co. and Sigma Chemical Co., respectively. These enzymes were used without further purification.

Enzymatic oxidative polymerization of *m*-substituted phenols. The following is a typical procedure for the polymerization (entry 7 in Table 1). Under air, *m*-cresol (0.54 g, 5.0 mmol) and HRP (1.0 mg) in a mixture of 12.5 mL of methanol and 12.5 mL of 0.1 M phosphate buffer (pH 7) were placed in a 50 mL flask. Hydrogen peroxide (5 % aq. solution, 3.4 mL, 5.0 mmol) was added dropwise to the mixture for 2 h at room temperature under air. After 3 h, the polymer precipitates were collected by centrifugation. The polymer was washed with an aqueous methanol (50:50 vol%), followed by drying in vacuo to give 0.52 g of the polymer (yield 97 %).

Measurements. SEC analysis was carried out using a TOSOH SC8010 apparatus with a refractive index (RI) detector at 40 °C under the following conditions: TSKgel G3000H_{HR} or G4000H_{HR} column with tetrahydrofuran (THF) eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. ^{1}H NMR spectra were recorded on a 270 MHz JEOL JNM-EX270J spectrometer. IR spectra were recorded on a Horiba FT-720 spectrometer. GC analysis was carried out using a Shimadzu GC-14B apparatus equipped with an FID detector and a TC-5 column (GL Sciences). DSC measurement was made at a 10 °C/min of heating rate under nitrogen using a Seiko SSC/5200 differential scanning calorimeter calibrated with an indium reference standard. TG analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry / differential thermal analysis at a heating rate of 10 °C/min under nitrogen in a gas flow rate of 300 mL/min.

Results and discussion

HRP-catalyzed polymerization of *m*-cresol. Previous reports showed that poly(*m*-cresol) obtained by the HRP-catalyzed polymerization in a mixture of

1,4-dioxane and buffer (80:20 vol%) was almost insoluble in common organic solvents and water.[5,6] Here, the author has performed the optimization to form a soluble polymer from *m*-cresol in a high yield by changing the solvent composition. The polymerization started with the addition of hydrogen peroxide, which was poured dropwise into the reaction mixture for 2 h. The reaction was carried out at room temperature under air. During the polymerization, the formation of pale yellow, powdery precipitates was observed. After 3 h, the precipitates were collected by centrifugation.

Table 1 summarizes the results of *m*-cresol polymerization catalyzed by HRP in an equivolume mixture of water-miscible organic solvent and buffer (0.1 M). The effect of the organic solvent was examined by using pH 7 phosphate buffer as a solvent (entries 1-4, 7, and 11). In all cases examined, the polymer was formed in high yields. The polymer obtained using ethanol, methanol and 2-propanol as cosolvent showed high solubility toward polar organic solvents such as acetone, DMF, DMSO, tetrahydrofuran (THF), and chloroform (entries 4, 7, and 11). In particular, the polymer obtained in the aqueous methanol (entry 7) was readily soluble in these solvents. On the other hand, the polymerization in using acetone, acetonitrile, 1,4-dioxane afforded the polymer which is partly soluble in such polar solvents (entries 1-3).

The buffer pH also affected the polymerization behaviors (entries 5-9). In the pH range from 4 to 9, the polymer was formed in good yields and the yield was the highest in the buffer pH of 7 (entry 7). On the other hand, no polymer formation was observed in the buffer of pH 10. In case of the HRP-catalyzed polymerization of phenol in a mixture of 1,4-dioxane and buffer (80:20 vol%), the yield in alkaline region (pH $\geq$ 9) was much lower than that in pH 7,[7] whereas the good yield was achieved in the pH range from 9 to 11 in the HRP-catalyzed polymerization of 2,6-dimethylphenol using a mixture of acetone and buffer (40:60 vol%) as solvent.[9] These data suggest that the polymer yield strongly depends on the solvent composition as well as the monomer structure. Distilled water also provided the soluble polymer in a high yield (entry 10).

Table 1. HRP-catalyzed polymerization of *m*-cresol. [a)]

Entry	Organic solvent	Buffer pH	Yield (%)	Mn x 10^{-2} [b)]	Mw/Mn [b)]
1	acetone	7	96	20 [c)]	7.0 [c)]
2	acetone	7	84	22 [c)]	2.5 [c)]
3	1,4-dioxane	7	87	27 [c)]	8.0 [c)]
4	ethanol	7	89	25	2.8
5	methanol	4	84	13	1.5
6	methanol	5	94	13	1.5
7	methanol	7	97	15	2.3
8	methanol	9	90	10	2.6
9	methanol	10	0		
10	methanol	distilled water	92	17	2.5
11	2-propanol	7	89	30	1.3

[a)] Polymerization of *m*-cresol (5.0 mmol) using HRP catalyst (1.0 mg) in an equivolume mixture of organic solvent and 0.1 M buffer (each 12.5 mL) at room temperature for 3 h under air. [b)] Determined by SEC using THF eluent. [c)] Data of the THF-soluble part.

The molecular weight of the polymer was estimated by size exclusion chromatographic (SEC) analysis using THF eluent. The molecular weight obtained in an aqueous methanol was lower than that in a mixture of other organic solvents and buffer. Among the mixed solvents to produce the soluble polymer, an aqueous 2-propanol afforded the highest molecular weight (entry 11). A similar tendency was observed in the peroxidase-catalyzed polymerization of bisphenol A in a mixture of organic solvent

and pH 7 phosphate buffer.[12]

Figure 1 shows effects of the mixing ratio of the buffer on the yield and the molecular weight of the polymer in a mixture of methanol and pH 7 phosphate buffer. There was a maximum point of the polymer yield in the buffer content of 50 %. The activity of HRP for the oxidation of phenol derivatives in the buffer was reported to be much higher than that in an aqueous organic solvent.[14] However, the yield of the polymer obtained in the buffer was much smaller than that in the aqueous methanol. One hypothesis is relevance of cluster formation by self-association of *m*-cresol[15] although complete explanation has not been given so far. In the range of buffer content from 30 to 80 %, the soluble polymer was obtained and the molecular weight decreased as increasing the buffer content because of the decrease in the solubility of the produced polymer.

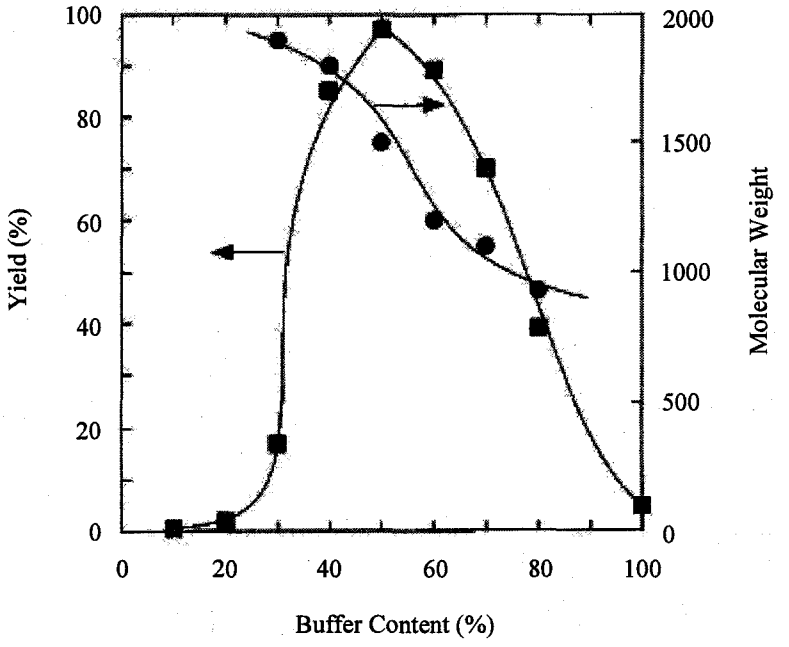


Figure 1. Effect of buffer content on the yield and molecular weight of polymer.

The effect of the enzyme amount was examined in an equivolume mixture of methanol and pH 7 phosphate buffer. When the HRP amount was 1 (entry 7) or 10 mg

per 5 mmol of *m*-cresol, the polymer was almost quantitatively obtained and the molecular weight and solubility scarcely changed. Assuming the molecular weight of HRP as $4x10^4$,[16] the total turnover of HRP is more than $2x10^5$, implying the extremely high catalytic activity of HRP for the present oxidative polymerization. In using the less amount (0.1 mg) of HRP, on the other hand, the polymer yield and molecular weight decreased (yield = 19 %; number-average molecular weight = 1100).

HRP- and SBP-catalyzed polymerization of *m*-substituted phenol derivatives. SBP was reported to be active as a catalyst for oxidative polymerization of phenol derivatives.[4,12,13,17] In a previous paper concerning peroxidase-catalyzed polymerization of bisphenol A,[12] SBP was superior to HRP for the efficient production of the polymer with higher molecular weight, whereas the yield of the polymer obtained from phenol by using SBP was lower than that by HRP under similar reaction conditions.[17] Moreover SBP has extremely high thermal stability and tolerate at lower pH than HRP.[18] Here, the enzymatic polymerization of *m*-substituted phenol derivatives was carried out by using HRP or SBP as a catalyst in an equivolume mixture of methanol and pH 7 phosphate buffer (Table 2). The catalytic activity of SBP used toward guaiacol oxidation was ca. quarter as large as that of HRP; four times amount of SBP was employed. In order to explore the effects of the monomer structure on the enzymatic polymerizability, substituent volume and HOMO level of the monomer were calculated by the Spartan set of programs using the AM1 method. The former is shown as an indication of steric factor toward the enzymatic polymerization. The latter is considered to strongly correlate the oxidizing reactivity.

The HRP-catalyzed polymerization of *m*-cresol afforded the polymer almost quantitatively and the yield enormously decreased in using *m*-ethylphenol. The polymer formation was not observed in the HRP-catalyzed polymerization of *m-iso*-propyl and *m-tert*-butylphenols. In case of the SBP-catalyzed polymerization of *m*-alkylphenols, on

the other hand, the polymer yield increased as a function of the substituent volume. Since the HOMO levels of *m*-alkylphenols are almost the same, the polymerization behavior was mainly explained by the relationships between the peroxidase origin and the steric factor of the monomer. It is thus to be noted that HRP has high catalytic activity toward the monomer having a smaller substituent, and SBP is preferable to the larger substituent monomer. This is probably due to the difference of the enzyme structure. SBP has a more solvent accessible δ-meso heme edge whereas it shows 57 % amino acid sequence identity;[19] therefore larger substrate would be favored than HRP. In case of the HRP-catalyzed polymerization of unbranched *p*-alkylphenols in the aqueous 1,4-dioxane, the polymer yield increased with increasing chain length of the alkyl group from 1 to 5, and the yield of the polymer from hexyl or heptylphenol was almost the same as that of the pentyl derivative.[20] Thus large substituents could be applied in the case of *p*-substituted phenol derivatives, whereas bulky *m*-substituted phenols were unfavorable in producing polymer in the HRP-catalyzed polymerization. In the peroxidase-catalyzed polymerization of *m*-cresol, chloro and bromophenols possessing almost the same volume substituent, the yield of the polymer by using HRP is larger than that by SBP, and in using both enzymes, *m*-cresol afforded the polymer in a higher yield than *m*-chloro and bromophenols. These results indicate that the monomers with a smaller (more negative) HOMO value afforded the polymer in a lower yield. A smaller HOMO value means the higher tolerance of the monomer to oxidation. The polymerization of *m*-methoxyphenol produced the polymer with molecular weight less than 1000.

Table 2. Peroxidase-catalyzed oxidative polymerization of *m*-substituted phenol derivatives. [a)]

Entry	Monomer			HRP [b)]			SBP [c)]		
	Substituent	Substituent volume [d)] ($\AA^3$)	HOMO [d)] (eV)	Yield (%)	Mn x 10^{-2} [e)]	Mw/Mn [e)]	Yield (%)	Mn x 10^{-2} [e)]	Mw/Mn [e)]
1	methyl	36	-9.02	97	15	2.3	49	11	3.5
2	ethyl	57	-9.02	37	14	1.8	72	12	2.5
3	isopropyl	77	-9.04	0			82	15	1.5
4	*tert*-butyl	98	-9.01	0			99	12	1.3
5	chloro	32	-9.30	58	12	1.4	10	19 [f)]	2.4 [f)]
6	bromo	39	-9.34	51	9.9	1.5	6	13	3.0
7	methoxy	47	-8.94	67	6.0	1.4	96	6.5	1.4
8	phenyl	104	-8.95	60	11	1.3	77	14	2.6

[a)] Polymerization of *m*-substituted phenol (5.0 mmol) using peroxidase catalyst in an equivolume mixture of organic solvent and 0.1 M buffer (each 12.5 mL) at room temperature for 3 h under air. [b)] Enzyme amount of 1.0 mg. [c)] Enzyme amount of 4.0 mg. [d)] Calculated by the Spartan set of programs using the AM1 method. [e)] Determined by SEC using THF eluent. [f)] Data of the THF-soluble part.

Structural analysis. In previous papers on the enzymatic polymerization of phenol and *p*-substituted phenols, NMR and IR analyses showed that the resulting phenolic polymers had a structure consisting of a mixture of phenylene and oxyphenylene units.[3,5-8,10,12,20] Here, the structure of poly(*m*-cresol) was analyzed by ^{1}H NMR and IR spectroscopies as well as titration of the phenolic hydroxy group in the polymer. In the IR spectrum of poly(*m*-cresol) (entry 7 in Table 1), a broad peak centered at 3500 cm^{-1} due to the vibration of O-H linkage of phenolic group, peaks at 1236 and 1192 cm^{-1} ascribed to the asymmetric vibrations of the C-O-C linkage and to the C-OH vibration, and a peak at 1105 cm^{-1} due to the symmetric vibration of the ether bond were observed. These data show that the enzymatically obtained polymer from *m*-cresol is also composed of a mixture of phenylene and oxyphenylene units. The phenolic polymers obtained by the HRP-catalyzed polymerization of phenol and *p*-alkylphenols show a small peak at ca. 1660 cm^{-1} due to the C=O stretching of the quinone, which may be formed by the oxidation of phenolic group at the polymer terminal. Interestingly, the present polymer has no peak due to the quinone carbonyl group. This is probably because *m*-substituent prevents the oxidation of phenolic group at the terminal.

^{1}H NMR spectrum shows three broad peaks due to the rigid structure: a peak centered at δ 8.4-10.0 due to the proton of phenolic group, a peak at δ 6.2-7.3 ascribed to the aromatic proton, and a peak at δ 1.4-2.5 due to the methyl proton. The integrated ratio of the latter two peaks is 5:6, indicating that the content of phenylene unit is 56 % based on the assumption that the degree of polymerization is 10, while the previous study showed that the phenolic polymer obtained from 2-(4-hydroxyphenyl)ethyl methacrylate had 70 % of phenylene unit. This should be related to the result that 1,4-linkage of phenolic polymer mainly consists of oxyphenylene unit and 1,2-linkage of it consists of phenylene unit. *m*-Substituted phenols have free *p*-position unlike *p*-substituted phenols and can form 1,4-linkage of oxyphenylene unit. This is why the

phenylene unit content of poly(*m*-cresol) is lower than that of poly(2-(4-hydroxyphenyl)ethyl methacrylate).

The unit content of residual phenolic group in the polymer was determined by conventional titration methods (Table 3) .[21] The phenylene unit content of poly(*m*-cresol) was 44 %, which is close to that determined by ^{1}H NMR. The effects of the monomer structure and enzyme type were small; the content values were not so different with each other. ^{1}H NMR analysis and titration of the residual phenolic group support that the enzymatically obtained poly(*m*-substituted phenol)s are of a mixture of phenylene and oxyphenylene structure.

Table 3. Contents of the phenylene unit in poly(*m*-substituted phenol)s. [a)]

Entry	Monomer	Enzyme	Content of phenylene unit (%)
1	*m*-cresol	HRP	44
2	*m*-cresol	SBP	43
3	*m*-ethylphenol	HRP	49
4	*m*-ethylphenol	SBP	46
5	*m*-isopropylphenol	SBP	56
6	*m-tert*-butylphenol	SBP	54
7	*m*-chlorophenol	HRP	50
8	*m*-methoxyphenol	HRP	59
9	*m*-methoxyphenol	SBP	51
10	*m*-phenylphenol	HRP	37
11	*m*-phenylphenol	SBP	37

[a)] Polymer preparation was as below: Polymerization of *m*-substituted phenol (5.0 mmol) using peroxidase catalyst in an equivolume mixture of organic solvent and 0.1 M buffer (each 12.5 mL) at room temperature for 3 h under air.

Polymerization profile. The polymerization in an equivolume mixture of methanol and a pH 7 phosphate buffer was monitored by using GC and SEC. Relationships between the monomer conversion and the polymer molecular weight are shown in Figure 2. The conversion gradually increased as a function of the added volume of hydrogen peroxide, whereas the molecular weight was almost constant during the polymerization. The monomer conversion was very close to the polymer yield (data not shown). These data indicate that there were no oligomers soluble in the reaction medium during the polymerization. This may be explained as follows: the resulting soluble dimer and oligomers reacted much faster than the monomer and the precipitated polymer was not reacted any more during the polymerization. This polymerization behavior is different from that of conventional oxidative polymerization in solution (condensation-type polymerization)[22] and of the enzymatic polymerization of 2,6-dimethylphenol and syringic acid showing gradual increase of molecular weight during the polymerization.[9,13]

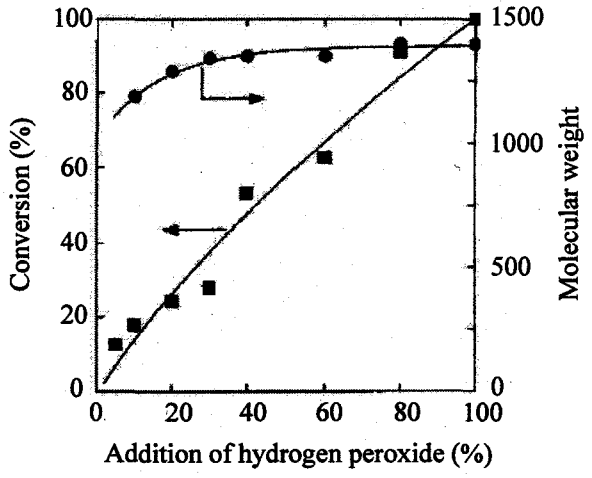
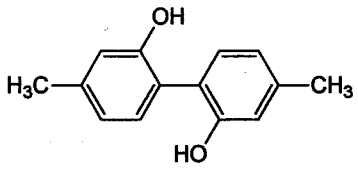
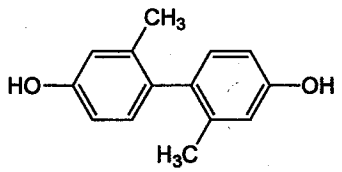
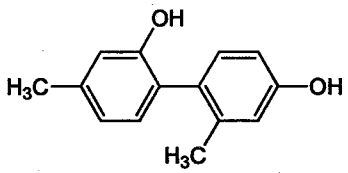
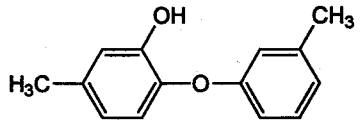
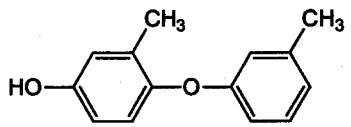


Figure 2. Addition of hydrogen peroxide versus monomer conversion and polymer molecular weight. The polymerization of *m*-cresol was carried out by using HRP (1.0 mg) as catalyst in an equivolume mixture of methanol and pH 7 phosphate buffer at room temperature under air. Hydrogen peroxide was added dropwise to the reaction mixture for 2 h.

The oxidation reactivity of *m*-cresol and its dimers was estimated by the HOMO level (Table 4). Based on the polymer structure, five dimers were postulated: carbon-carbon linked dimers (entries 2-4) and carbon-oxygen linked dimers (entries 5 and 6). All the dimers calculated showed the larger HOMO level than *m*-cresol, supporting the higher reactivity of the dimers toward oxidation. Calculated heat of formation for the dimers showed that C-C linkage was preferable in the polymerization

Table 4. HOMO level and heat of formation of *m*-cresol and its dimers. [a)]

Entry	Compound	HOMO (eV)	Heat of formation (kJ/mol)
1	*m*-cresol	-9.02	-124
2		-8.83	-233
3		-8.81	-220
4		-8.84	-227
5		-8.90	-152
6		-8.70	-145

[a)] Calculated by the Spartan set of the programs using AM1 method.

of *m*-cresol, indicating not only thermodynamic factor but kinetic one probably due to the difference of the spin density at each atom of a phenoxy radical and/or interactions between the radical molecules would affect the coupling behavior.[23,24]

Thermal properties. Thermal properties of the present phenolic polymers were evaluated by differential scanning calorimetry (DSC) and thermogravimetry (TG). DSC measurement was carried out under nitrogen and the glass transition temperature (Tg) was determined in the second or third scan. Figure 3 shows DSC charts of the polymers from *m*-cresol, *m*-ethyl and *m-tert*-butylphenols. For all the polymers from *m*-alkylphenols examined, Tg was observed (Table 5). Poly(*m*-cresol) showed Tg at higher than 200 °C. In case of the polymers from *m*-alkylphenols, the Tg value decreased with increasing the bulkiness of the substituent.

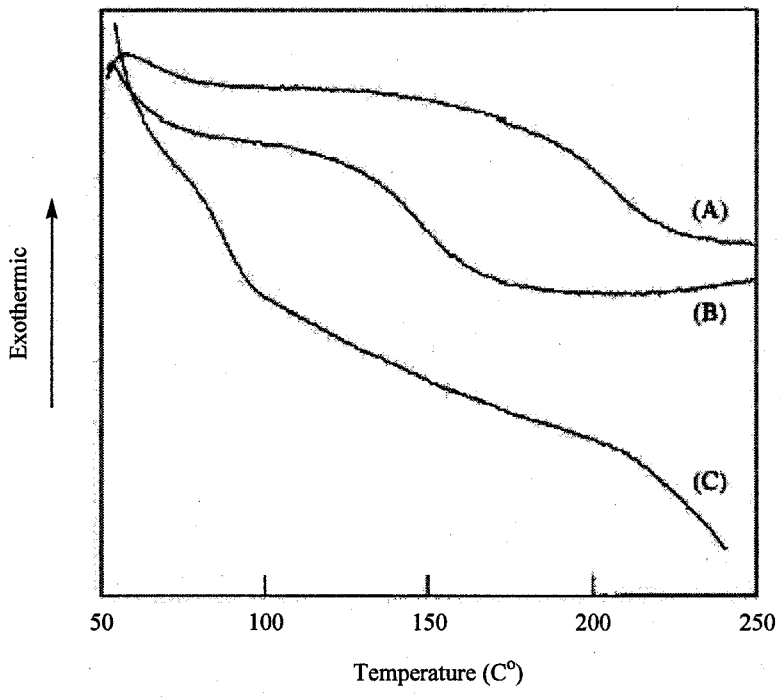


Figure 3. DSC traces of (A) poly(*m*-cresol), (B) poly(*m*-ethylphenol), and (C) poly(*m-tert*-butylphenol).

Table 5. Thermal properties of poly(*m*-substituted phenol)s. [a)]

Entry	Polymerization		Property		
	monomer	enzyme	T_g [b)] (°C)	T_{d5} [c)] (°C)	residue [d)] (%)
1	*m*-cresol	HRP	204	336	42
2	*m*-cresol	SBP	202	303	40
3	*m*-ethylphenol	SBP	150	325	24
4	*m*-isopropylphenol	SBP	127	307	13
5	*m*-*tert*-butylphenol	SBP	90	231	8
6	*m*-chlorophenol	HRP	---[e)]	195	34
7	*m*-bromophenol	HRP	183	216	32
8	*m*-methoxyphenol	SBP	---[e)]	272	47
9	*m*-phenylphenol	SBP	94	221	34

[a)] Polymer preparation was as below: Polymerization of *m*-substituted phenol (5.0 mmol) using peroxidase catalyst in an equivolume mixture of organic solvent and 0.1 M buffer (each 12.5 mL) at room temperature for 3 h under air. [b)] Grass transition temperature determined by DSC under nitrogen at a heating rate of 10 °C/min. [c)] Temperature at 5 % weight loss determined by TGA under nitrogen at a heating rate of 10 °C/min. [d)] Content of residue in wt.-% at 1000 °C determined by TGA under nitrogen at a heating rate of 10 °C/min. [e)] Not detected.

Tg of poly(*p*-alkylphenol)s obtained by the HRP-catalyzed polymerization in an aqueous 1,4-dioxane has been measured already.[20] Most of them showed no clear Tg and only poly(*p*-*tert*-butylphenol) showed Tg of 182 °C. On the other hand, Tg was observed in the DSC chart of poly(*m*-alkylphenol)s and Tg of poly(*m*-*tert*-butylphenol)

was much lower than that of *p*-isomer.

Thermal stability was evaluated by TG measurement under nitrogen. The decomposition behavior of poly(*m*-substituted phenol)s was similar to those obtained from phenol and *p*-substituted phenols.[6-8,12,17,20] Among the polymers examined, poly(*m*-cresol) had the highest temperature at 5 weight % loss (T_{d5}) and the residual weight of poly(*m*-methoxyphenol) at 1000 °C was the largest (Table 5). In the case of poly(*m*-alkylphenol)s, T_{d5} and residual weight at 1000 °C decreased as a function of the bulkiness of the substituent.

Conclusion

Peroxidases, HRP and SBP, efficiently catalyzed oxidative polymerization of *m*-substituted phenols. Under appropriate reaction conditions, a new class of phenolic polymers showing high solubility toward common polar solvents were obtained in high yields. By changing the solvent composition, the polymer molecular weight and solubility could be controlled. The polymerization behaviors strongly depended on the enzyme type and monomer structure; HRP could readily polymerize monomers having small substituents, whereas in the case of monomers having large substituents, the high yield was achieved by using SBP catalyst. The resulting polymer was composed of a mixture of phenylene and oxyphenylene units. Thermal analysis showed that poly(*m*-cresol) had relatively high thermal stability.

References

1) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*,

31.

2) Akkara, J. A.; Senecal, K. J.; Kaplan, D. L. *J. Polym. Sci., Polym. Chem. Ed.* **1991**, *29*, 1561.

3) Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, 423.

4) Wang, P.; Martin, B. D.; Parida, S.; Rethwisch, D. G.; Dordick, J. S. *J. Am. Chem. Soc.* **1995**, *117*, 12885.

5) Kurioka, H.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1994**, 15, 507.

6) Uyama, H.; Kurioka, H.; Komatsu, I.; Sugihara, J.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* 1995, **68**, 3209 (1995)

7) Uyama, H.; Kurioka, H.; Sugihara, J.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 189.

8) Kobayashi, S.; Uyama, H.; Kurioka, H. *Macromol. Rapid Commun.* **1996**, *17*, 503.

9) Ikeda, R.; Sugihara, J.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 8702.

10) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.

11) Ayyagari, M.; Akkara, J. A.; Kaplan, D. L. *ACS Symp. Ser.* **1998**, *684*, 112.

12) Kobayashi, S.; Uyama, H.; Ushiwata, T.; Uchiyama, T.; Sugihara, J.; Kurioka, H. *Macromol. Chem. Phys.* **1998**, *199*, 777.

13) Ikeda, R.; Sugihara, J.; Uyama, H.; Kobayashi, S. *Polym. International* **1998**, *47*, 295.

14) Ryu, K.; Dordick, J. S. *Biochemistry* **1992**, *31*, 2588.

15) Oguchi, T.; Wakisaka, A.; Tawaki, S.; Tonami, H.; Uyama, H.; Kobayashi, S. *J. Phys. Chem. B* **2002**, *106*, 1421.

16) Saunders, B. C.; Holmes-Siedle, A. G.; Stark, B. P. In *Peroxidase*;

Buttersworth: London, 1964.

17) Uyama, H.; Kurioka, H.; Komatsu, I.; Sugihara, J.; Kobayashi, S. *Macromol Reports* **1995**, *A32*, 649.

18) McEldoon, J. P.; Pokora, A. R.; Dordick, J. S. *Enzyme Microb. Technol.* **1995**, *17*, 359.

19) Henriksen, A.; Mirza, O.; Indiani, C.; Teilum, K.; Smulevich, G.; Welinder, K. G.; Gajhede, M. *Protein Science* **2001**, *10*, 108.

20) Uyama, H.; Kurioka, H.; Sugihara, J.; Komatsu, I.; Kobayashi, S. *J. Polym. Sci., Polym. Chem. Ed.* **1997**, *35*, 1453.

21) Asakura, K.; Shiotani, T.; Honda, E.; Matsumura, S. *J. Jpn. Oil. Chem. Soc.* **1993**, *42*, 656.

22) Koch, M.; Heitz, W. *Makromol. Chem.* **1983**, *184*, 779.

23) Musso, H. In *Oxidative Coupling of Phenols*; Taylor, W. I.; Battersby, A. R. Eds.; Marcel Dekker Inc.: New York, 1967.

24) Armstrong, D. R.; Cameron, C.; Nonhebel, D. C.; Perkins, P. G. *J. Chem. Soc. Perkin Trans. II* **1983**, 563.

Chapter 2

Chemoselective Oxidative Polymerization of *m*-Ethynylphenol by Peroxidase Catalyst to a New Reactive Phenolic Polymer

Introduction

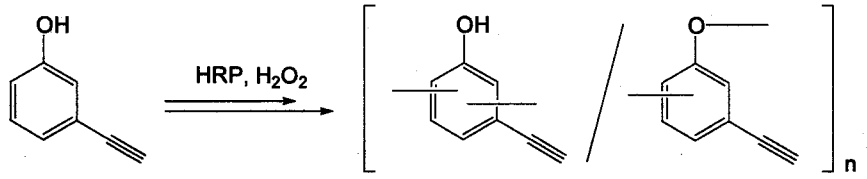
In the field of polymer chemistry, an acetylenic moiety is very useful as a polymerizable group toward transition metal catalysts[1,2] and also as a crosslinkable group for thermal curings.[3] Its polymerization produces a poly(acetylene), whose film has functions such as high electroconductivity and selective permeability. The curing through the group is often suitable for applications of electronic devices, because the curing group does not contain a heteroatom causing high polarity.[4]

Chemoselective polymerization of a monomer having more than two polymerizable (reactive) groups is expected to afford a new class of reactive polymer having polymerizable or crosslinkable groups in the side chain. In case of such a monomer having an unsaturated polymerizable group, however, it is often difficult to achieve the chemoselective polymerization without involving reaction of the unsaturated group because of high reactivity toward various polymerization catalysts. Very recently, it has been reported that in the peroxidase-catalyzed oxidative polymerization of 2-(4-hydroxyphenyl)ethyl methacrylate possessing two polymerizable groups, phenolic and methacryloyl groups, only the phenolic moiety was chemoselectively polymerized to produce a polymer having methacryloyl group in the side chain,[5] suggesting specific catalysis of the enzyme.

In using conventional oxidation catalysts such as a copper/amine system, aromatic compounds having two ethynyl groups are known to be subjected to oxidative

coupling, yielding poly(diaceytylene)s[6] by Glacer reaction. The copper/amine catalyst is also very effective for oxidative polymerization of 2,6-substituted phenols to give poly(1,4-oxyphenylene)s.[7-8] Therefore, poly(1,4-oxyphenylene) having ethynyl group in the side chain can not be synthesized directly by the oxidative polymerization using the copper/amine catalyst; such polymer was obtained by the polymerization of a 2,6-disubstituted phenol having silyl-protected ethynyl group and the subsequent removal of the silyl group.[9] The present chapter deals with peroxidase-catalyzed polymerization of *m*-ethynylphenol, an acetylene group-containing phenol (**1**) (Scheme 1), and the following thermal curing of the polymer. The author has found that the phenolic moiety of **1** was chemoselectively polymerized by using horseradish peroxidase (HRP) as a catalyst to give the polymer having ethynyl group in the side chain, which is applicable to production of pyropolymer in high carbon yield. Pyrolysis of polymers is often used to prepare grafite-like materials possessing high electrical properties.[10,11]

Scheme 1.



1 **2**

Experimental section

Materials. *m*-Ethynylphenol was synthesized according to the literature.[12] Horseradish peroxidase (HRP) (EC 1.11.1.7, 100U/mg) was purchased from Wako Pure Chemical Co. and used without further purification. All other reagents were commercially available and used as received.

Enzymatic reaction of 1. Monomer **1** (0.30 g, 2.5 mmol) and HRP (2.0 mg) in a mixture of 6.25 mL of methanol and 6.25 mL of 0.1 M phosphate buffer (pH 7) were placed in a 50 mL flask. Hydrogen peroxide (5 % aq. solution, 1.7 mL, 2.5 mmol) was added dropwise under gentle stirring to the mixture for 2 h at room temperature under air. After 3 h, polymer precipitates were collected by centrifugation. The polymer was washed with an aqueous methanol (50:50 vol%), followed by drying in vacuo to give 0.28 g of the polymer (yield 95 %).

Enzymatic treatment of phenylacetylene. Reaction of phenylacetylene (2.5 mmol) using HRP (1.0 mg) catalyst was carried out in a mixture of 8.75 mL of 1,4-dioxane and 3.75 mL of 0.1 M phosphate buffer (pH 7.0). Hydrogen peroxide (5.0 % aq. solution, 1.7 mL, 2.5 mmol) was added dropwise for 2 h. During the reaction, polymeric precipitates were not formed. In the HRP-catalyzed treatment of phenylacetylene (2.5 mmol) and phenol (1.25 mmol) under the similar reaction conditions, black powdery materials were precipitated, which were collected by centrifugation, followed by washing with an aqueous 1,4-dixoane to give 0.089 g of the polymer (75 % yield based on phenol).

Copper/amine-catalyzed oxidation of 1 and phenylacetylene. Reaction of **1** (2.5 mmol) using copper chloride (I) (0.30 mmol) / *N,N,N',N'*-tetraethylethylenediamine (TEED) (0.90 mmol) was performed in 10 mL of dimethoxyethane at room temperature for 24 h under air. The mixture was concentrated under reduced pressure and the residue was washed with 50 mL of water containing 2 mL of concentrated hydrochloric acid to give 0.24 g of bis(3-hydroxyphenyl)butadiyne (**3**) (yield 81 %): ^{1}H NMR ($CDCl_3$): δ 6.89 (dd, 2H, Ar), 6.94 (dd, 2H, Ar), 7.02 (dd, 2H, Ar), 7.23 (dd, 2H, Ar); IR (neat): 3200~3300 (υ O-H), 2150 cm^{-1} (υ carbon-carbon triple bond). In the reaction of phenylacetylene (2.5 mmol, 0.26 g) or the coreaction of phenylacetylene (2.5 mmol,

0.26 g) and phenol (1.25 mmol, 0.12 g) under the similar conditions, 0.19 g (74 % yield) or 0.18 g (72 % yield based on phenylacetylene) of diphenylbutadiyne (**4**) was obtained by the similar isolation procedures: ^{1}H NMR ($CDCl_3$): δ 7.38 (m, 6H, Ar), 7.55 (dd, 4H, Ar); IR (neat): 2110 cm^{-1} (υ carbon-carbon triple bond).

Heat treatment. Poly(*m*-ethynylphenol) (PEP) was heated at a heating rate of 10° C/min to reach predetermined temperatures and treated for further 30 min at the temperature under nitrogen.

Measurement. SEC analysis was carried out using a TOSOH SC8010 apparatus with a refractive index (RI) detector at 40 °C under the following conditions: TSKgel G3000H_{HR} column and THF eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. NMR spectra were recorded on a 400 MHz Bruker DPX-400 spectrometer. IR spectra were recorded on a Horiba FT720 spectrometer. TG analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry / differential thermal analysis at a heating rate of 10 °C/min in a gas flow rate of 300 mL/min. Raman spectra were excited by using a 514.5 nm line (50 mW) of an argon ion laser (NEC, GLG3280). The scattered light was collected in a backscattering (180°) geometry. The spectra were recorded using a spectrometer (Jobin-Yvon, T64000) equipped with a multi-channel CCD detector. For each measurement the integration time was 300 s. WAXD patterns were recorded on a Rigaku RINT-1400 (40kV/200mA) system with the Cu-Kα X-ray beams.

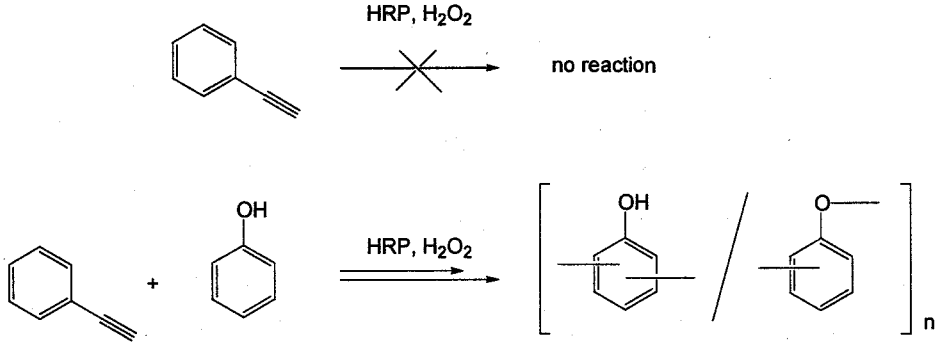
Results and discussion

HRP-catalyzed polymerization of *m*-ethynylphenol. The HRP-catalyzed polymerization of **1** was carried out using hydrogen peroxide as an oxidizing agent in methanol/phosphate buffer (pH 7.0) (50:50 vol%) at room temperature under air. During the polymerization, powdery materials were formed. After 3 h, the resulting polymer was collected by centrifugation (95 % yield). Polymer **2** was soluble in acetone, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), pyridine, and tetrahydrofuran (THF), partly soluble in chloroform, and insoluble in hexane and water. The molecular weight and polydispersity were determined by size exclusion chromatography to be 1700 and 1.8, respectively. In the reaction without HRP (control experiment), the monomer was recovered unchanged, indicating that the present polymerization took place through the enzyme catalysis.

The polymer structure was confirmed by ^{1}H NMR and IR spectroscopies. In the ^{1}H NMR spectrum of **2**, observed were three broad large peaks at δ 3~4, 6~8, and 9~10, ascribable to acetylenic, aromatic, and phenolic hydroxy protons, respectively. FT-IR spectrum of **2** shows characteristic peaks at 3290 and 2100 cm^{-1} due to the vibration of the carbon-hydrogen and carbon-carbon bonds of the ethynyl group. These data indicate that the resulting polymer possessed the ethynyl group in the side chain. The previous study on the HRP-catalyzed oxidative polymerization of *m*-alkylphenols showed that the polymer was of a mixed structure of phenylene and oxyphenylene units. The peaks' pattern of FT-IR spectrum of the present polymer is very similar to that of enzymatically synthesized poly(*m*-alkylphenol)s,[13] suggesting the formation of the polymer consisting of phenylene and oxyphenylene units from **1**. The ratio of phenylene and oxyphenylene units in polymer **2** was calculated to be ca. 1:1 from the amount of the residual phenolic group determined by conventional titration methods.[14]

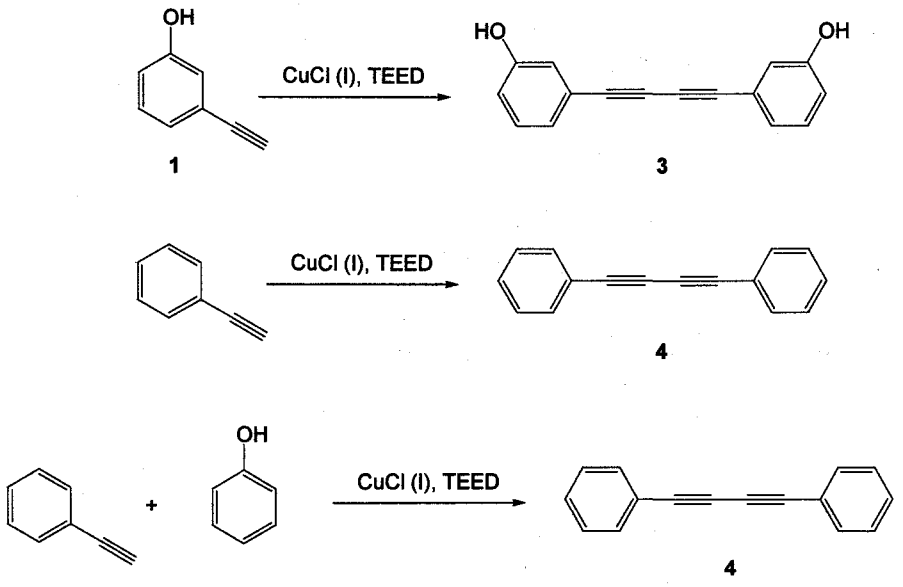
Verification of chemoselectivity. In order to examine the chemoselectivity between phenolic and ethynyl groups toward HRP catalyst, the following model reactions were carried out (Scheme 2): the reaction of phenylacetylene catalyzed by HRP and the HRP-catalyzed copolymerization of phenol and phenylacetylene.[12] Polymer formation was not observed in the former reaction. The latter produced the polymer containing no ethynyl group.[13]

Scheme 2.



For reference, the polymerization of **1** using the copper chloride (I) / TEED catalyst was examined. The phenolic polymer was not formed; the coupling of the acetylene moiety took place to give selectively bis(3-hydroxyphenyl)butadiyne (**3**) in 81 % yield (Scheme 3).[14] Instead of HRP, the above model reactions were carried out using the copper/amine catalyst. In both cases, only diphenylbutadiyne (**4**) was exclusively obtained in ca. 75 % isolated yield. These data clearly indicate the chemoselective catalysis of HRP for the oxidative polymerization of **1**.

Scheme 3.



Thermal analysis of 2. The thermal properties of **2** was evaluated by thermogravimetry (TG) under nitrogen. Figure 1 shows TG traces of **2** and poly(*m*-cresol), which were enzymatically synthesized under the similar reaction conditions.[11] In case of poly(*m*-cresol), rapid weight decrease was observed around 400 °C. Conventional phenol resins show the similar degradation behaviors. On the other hand, thermal degradation of **2** proceeded slowly. It is known that thermal treatment of phenolic polymers at high temperature (>400 °C) produces carbonized polymers.[15,16] At such a temperature, the residual ratio of **2** was much higher than that of poly(*m*-cresol), indicating that **2** was a very good precursor of the carbonized materials. This is probably because the ethynyl group was reacted at the initial stage of TG measurement to give the crosslinked polymer showing less thermal degradability.

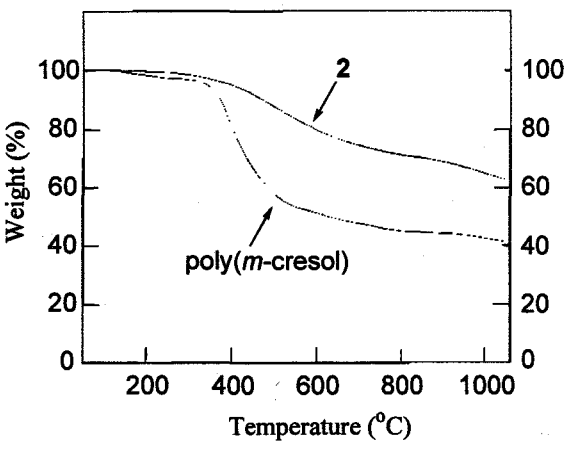


Figure 1. TG traces of **2** and poly(*m*-cresol) under nitrogen.

The present polymer was cured even at 50 °C to give the insoluble polymer. DSC profile (data not shown) shows broad exothermic peak around 200 °C. Furthermore, the peak due to the ethynyl group in the FT-IR spectrum completely disappeared at 200 °C. From these data, the ethynyl group in **2** possessed high reactivity for thermal curing.

The present polymer was separately heated at 450 °C, 650 °C, and 850 °C for 30 min under nitrogen (PEP-450, PEP-650, PEP-850). After cooling down to room temperature, black powders were obtained. IR spectra of them show no characteristic absorbance of ethynyl group at 3290 and 2100 cm^{-1} unlike **2**, indicating participation of the ethynyl group in the curing process (Figure 2). The pyropolymer obtained by heating at 850 °C showed no absorbance in the IR spectrum. This would be because the polymer was gradually forming graphite-like structure and its high symmetry led to no absorbance of the IR spectrum.

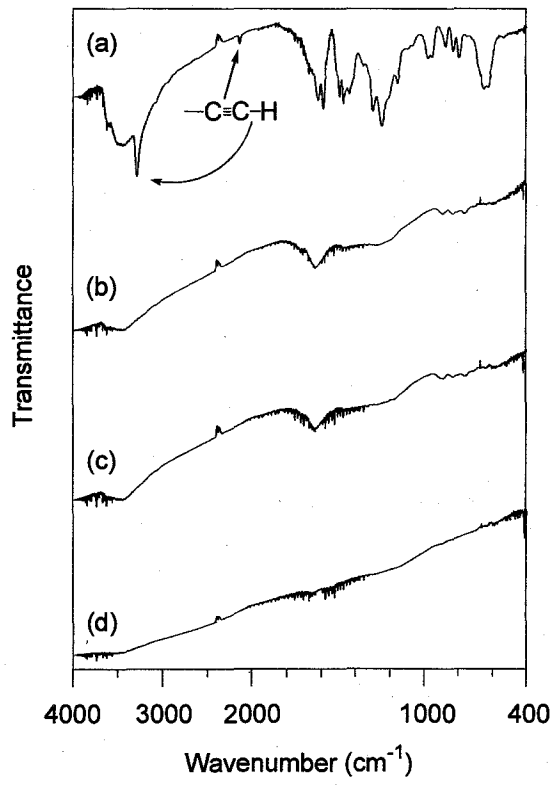


Figure 2. FT-IR spectra of (a) PEP, (b) PEP-450, (c) PEP-650, and (d) PEP-850.

The Raman spectra show two broad bands at 1350 cm^{-1} and 1600 cm^{-1} for all pyropolymers (Figure 3). A graphite-like structure has also been known to show distinct bands at 1350 cm^{-1} and 1630 cm^{-1}.[17] The former corresponds to the A_{1g} vibration mode, which becomes Raman active unless the graphite crystal has infinite size as a result of the relaxation of symmetry selection rules, the latter to the E_{2g} vibration mode due to the structural defect of the graphite lattice. These results indicate that the pyropolymers consist of small crystallites of graphite and have disordered graphite structure.

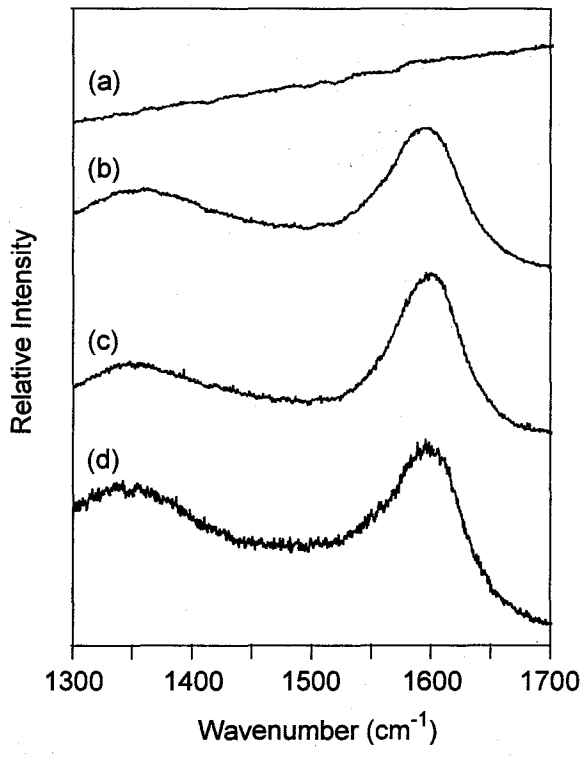


Figure 3. Laser-Raman spectra of (a) PEP, (b) PEP-450, (c) PEP-650, and (d) PEP-850.

Powder X-ray diffraction analysis was carried out on the pyropolymers to check the crystallinity (Figure 4). In all cases, observed bands were broad, showing that the samples were amorphous and the interlayer distance is around 4 Å, which is longer than that of graphite (3.35 Å). These results are consistent with the disordered graphite-like structure proposed from the Raman spectra.

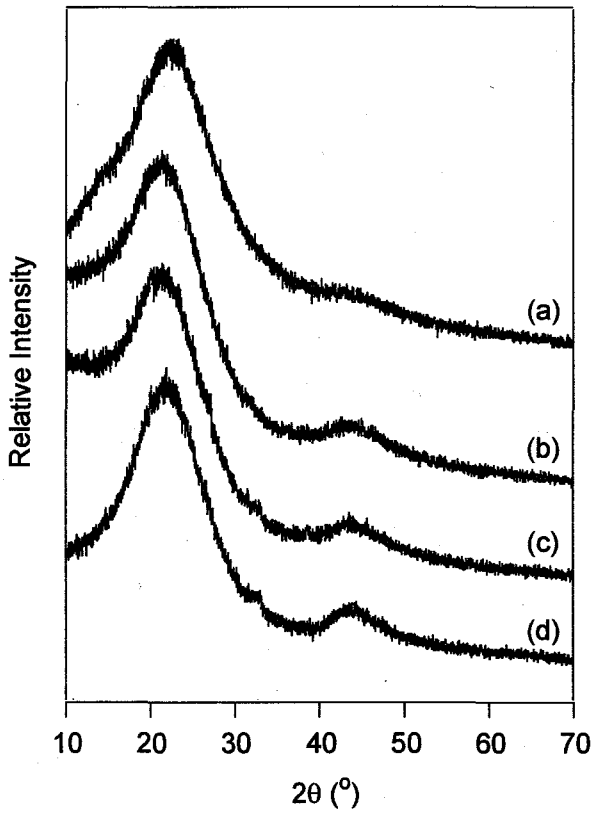


Figure 4. Powder X-ray diffraction pattern of (a) PEP, (b) PEP-450, (c) PEP-650, and (d) PEP-850.

A model structure of a moluculer-order graphite-like material is given by Tanaka et al (Figure 5).[10] Spectroscopic analyses show that the present polymer has the structure similar to it.

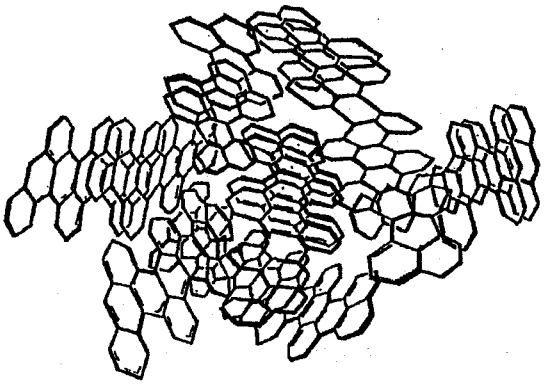


Figure 5. An illustrative model structure of graphite-like material.

Conclusion

The phenolic moiety was chemoselectively reacted in the HRP-catalyzed polymerization of **1** in an aqueous methanol. The resulting polymer possessed the ethynyl group in the side chain, and hence, is expected to have various potential applications as a highly reactive starting polymer. Furthermore, the residual ratio of **2** at a high temperature (~ 1,000 °C) was higher than that of the other phenolic polymers, suggesting many possibilities for new precursors of functional carbonized materials. The analysis of the curing process showed that the present polymer formed graphite-like amorphous carbon by heating, which is expected to have high electrical properties. The present polymer is synthesized without use of toxic formaldehyde under mild reaction conditions and cured at low temperature. Therefore, the present method is an environmentally benign process of functional crosslinked polymer production, giving an example system of green polymer chemistry.

References

1) Masuda, T.; Higashimura, T. *Adv. Polym. Sci.* **1992**, *81*, 121.

2) Masuda, T. In *Catalysis in Precision Polymerization*; Kobayashi, S., Ed.; John Wiley & Sons: Chichester, 1997; Chap. 2.

3) Taguchi, Y.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1995**, *16*, 183.

4) Taguchi, Y.; Uyama, H.; Kobayashi, S.; Osada, K. In *Macromolecular Engineering: Contemporary Themes*, Mishra, M. K.; Nuyken, O.; Kobayashi, S.; Yagci, Y.; Sar, B., Eds.; Plenum Press: New York, 1995; p319-328.

5) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules*

1998, *31*, 554.

6) Naarmann, H. In *The Polymeric Materials Encyclopedia*, Salamone, J. C., Ed.; CRC Press: Boca Raton, 1996; p4852-4864.

7) Hay, A. S; Blanchard, H. S.; Endres, G. F.; Eustance, J. W. *J. Am. Chem. Soc.* **1959**, *81*, 6335.

8) Hay, A. S. *J. Polym. Sci., Polym. Chem. Ed.* **1998**, *36*, 505.

9) Yang, H.; Hay, A. S. *J. Macromol. Sci.-Pure Appl. Chem.* **1994**, *A31*, 155.

10) Tanaka, K.; Yata, S.; Yamabe, T. *Synthetic Metals* **1995**, *71*, 2147.

11) Kim, D. S.; Suh, M. C.; Shim, S. C. *Synthetic Metals* **1996**, *80*, 291.

12) Kwock, E. W.; Baird, T., Jr.; Miller, T. M. *Macromolecules* **1993**, *26*, 2935.

13) Tonami, H.; Uyama, H.; Kobayashi, S.; Kubota, M. *Macromol. Chem. Phys.* **1999**, *200*, 2365.

14) Oguchi, T.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1999**, *20*, 401.

15) Uyama, H.; Kurioka, H.; Sugihara, J.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 189.

16) Yamashita, Y; Ouchi, K. *Carbon* **1981**, *19*, 89.

17) Suh, M. C.; Shim, S. C. *Macromolecules* **1995**, *28*, 8707.

Chapter 3

Enzymatic Polymerization of *p*-Substituted Phenol Derivatives: Synthesis of Poly(hydroquinone) and Poly(tyrosine)

Introduction

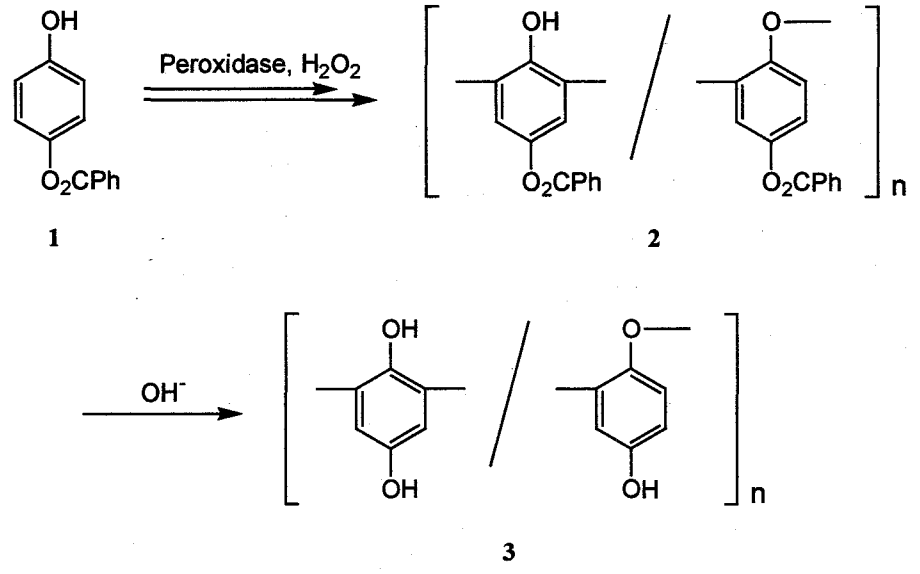
There are several papers reported on the enzymatic synthesis of reactive phenolic polymers. In the peroxidase-catalyzed polymerization of a phenol derivative having methacryloyl group, the phenolic moiety was chemoselectively polymerized to give a polymer having the methacryloyl group in the side chain.[1] The polymer was subjected to thermal and photo crosslinking, yielding the insoluble product. A phenolic polymer containing a thymidine pendant group was synthesized by using peroxidase catalyst.[2]

Redox-active polymers possess various applications for batteries, sensors, electrical conductors, and antioxidants.[3] Poly(hydroquinone) is one of the most typical redox polymers. Direct oxidative reaction of hydroquinone using enzyme catalyst produces benzoquinone. On the other hand, electrochemical polymerization of hydroquinone produced poly(1,4-dihydroxy-2,5-phenylene).[4] Another approach of poly(hydroquinone) synthesis was reported: the enzymatic oxidative polymerization of glucose-β-D-hydroquinone and subsequent acid hydrolysis of the resulting polymer produced poly(1,4-dihydroxy-2,6-phenylene).[5] The former was insoluble in polar organic solvents, whereas the latter was soluble in polar organic solvents. The latter behaved as a redox mediator for glucose sensing.[6]

Most of the enzymatically synthesized phenoic polymers have a mixed structure of phenylene and oxyphenylene units.[7,8] This chapter deals with synthesis of a

new poly(hydroquinone) derivative (**3**) by the peroxidase-catalyzed polymerization of 4-hydroxyphenyl benzoate (**1**) and the subsequent hydrolysis of the resulting polymer (Scheme 1). The structure of **3** was different from that from glucose-β-D-hydroquinone.

Scheme 1.



Amino acid-based polymers including polypeptides have been remarkably developed owing to their wide potential application for biocompatible materials as well as useful chemical materials.[9] Some of these polymers have unique properties and functions derived from amino acid moiety. Modification and functionalization of natural proteins have been extensively studied in the standpoint of materials development from renewable resources. A thermally polymerized product from aspartic acid has received much attention as a new useful class of biodegradable, water-soluble polymeric materials.[10] The polymers consisting of the amino acid moiety in the main chain often showed good biodegradability. Recently, multiblock copolymers of GlyAlaGlyAla and poly(ethylene oxide) were prepared as a model of silk-based materials, which formed nanostructures through β-sheet self-assembly.[11] Polymerization of vinyl monomers possessing the amino acid group has been also extensively investigated.[12]

Peroxidases induce the reticulation of extension, which is a maior protein in the cell wall. Recently the dimerization of calmodulin via a dityrosine bridge by peroxidases was analyzed.[13] In the case of proteins, the degree of polymerization is generally limited to trityrosine because of steric hindrance. The present chapter also deals with enzymatic polymerization of a tyrosine derivative, in which a phenol moiety was subjected to an oxidative coupling. The polymerization of tyrosine esters, followed by alkaline hydrolysis of the ester group, produced poly(tyrosine) having amino acid moiety in the side chain.

In chapters 1 and 2, enzymatic polymerization of *m*-substituted phenol derivatives was introduced. In this chapter, peroxidase-catalyzed polymerization of *p*-substituted phenols is focused.

Experimental part

Materials. Horseradish peroxidase (EC 1.11.1.7, 100U/mg) and soybean peroxidase (EC 1.11.1.7, 60U/mg) were purchased from Wako Pure Chemical Co. and Sigma Chemical Co., respectively. D-Tyrosine methyl ester hydrochloride was synthesized according to the literature.[14] Enzymes and other reagents were commercially available and used as received.

Synthesis of 4-hydroxyphenyl benzoate.[15] Benzoyl chloride (7.0 g, 50 mmol) was added dropwise to a solution of hydroquinone (5.5 g, 50 mmol) and sodium carbonate (6.0 g, 55 mmol) in 20 mL of water under nitrogen. The mixture was stirred below a temperature of 20 °C. After 5 min of stirring, the obtained residue was filtered and poured into 200 mL of methylene chloride. The solution was dried over $MgSO_4$ and the solvent was removed in vacuo. The crude product was recrystallized from carbon

tetrachloride to give 10 g of 4-hydroxyphenyl benzoate (yield 93 %). m.p.: 130-131 °C.

Enzymatic reaction. The following is a typical procedure for the polymerization (entry 6). Under air, 4-hydroxyphenyl benzoate (1.0 mmol) and soybean peroxidase (4.0 mg) in an equivolume mixture of 0.1 M phosphate buffer (pH 7.0) and 1,4-dioxane (10 mL) were placed in a 50 mL flask. Hydrogen peroxide (5.0 % aq. solution, 0.75 mL, 1.1 mmol) was added dropwise to the mixture for 2 h at room temperature under air. After 3 h, polymer precipitates were collected by centrifugation. The polymer was washed with an aqueous methanol (50:50 vol%), followed by drying in vacuo to give 0.19 g of the polymer (yield 87 %).

Hydrolysis of polymer 2. The polymer (0.50 g) was dissolved in a mixed solvent of tetrahydrofuran (8.0 mL) and water (2.0 mL) containing potassium hydroxide (40 mmol). The mixture was kept at 60 °C for 24 h. The layer of the organic solvent was removed, followed by the addition of 100 mL of 1.0 M HCl to the redisue. The formed precipitates were separated by centrifugation. The polymer was washed with water and dried in vacuo to give 0.16 g of the hydrolyzed polymer (yield 62 %).

HRP-catalyzed polymerization of tyrosine ester hydrochlorides. A typical run was as follows (entry 3 in Table 1). L-Tyrosine ethyl ester hydrochloride (0.98 g, 4.0 mmol) and HRP (10 mg) in 1.0 M Tris buffer of pH 7.6 (25 mL) were placed in a 50 mL flask. Hydrogen peroxide (5.0% aqueous solution, 2.7 mL, 4.0 mmol) was added dropwise to the mixture for 2 h at room temperature under air. After 3 h, polymer precipitates were collected by centrifugation. The polymer was washed with water, followed by drying in vacuo to give 0.80 g of the polymer (yield 82%). ^{1}H NMR (DMSO-*d6*): δ 1.0-1.5 (br, CH_3), 2.7-3.2 (br, $ArCH_2$), 3.7-4.0 (br, CH), 4.0-4.2 (br, OCH_2), 6.5-7.5 (br, Ar); IR (KBr): 3200-3400 (n(O-H)), 1731 (ν(C=O)), 1608, 1506

(ν(C=C) of Ar), 1214 (ν(C(Ar)-O-C(Ar) and C(Ar)-OH)), 1028 (ν(C(Ar)-O-C(Ar))).

Alkaline hydrolysis of poly(tyrosine ethyl ester). Poly-(L-tyrosine ethyl ester) (1.0 g) was kept in 1.0 M NaOH solution (25 mL) at 60 °C. After 24 h, the mixture was neutralized with 6.0 M hydrochloride solution, and subsequently, the polymer was purified by dialysis (cutoff molecular weight 100). The remaining solution was lyophilized to give 0.28 g of the polymer (yield 40%). ^{1}H NMR (D_2O): δ 2.7-3.2 (br, $ArCH_2$), 3.6-4.0 (br, CH), 6.5-7.5 (br, Ar).

Measurements. In the study of poly(hydroquinone), SEC analysis was carried out using a TOSOH SC8010 apparatus with a refractive index (RI) detector at 40 °C under the following conditions: TSKgel G3000H_{HR} column and THF eluent at a flow rate of 1.0 mL/min. In the study of poly(tyrosine), SEC analysis was mainly carried out using a TOSOH SC8020 apparatus with a refractive index (RI) detector at 60 °C under the following conditions: TSKgel α3000 column and DMF containing 0.10 M LiCl eluent at a flow rate of 0.5 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. ^{1}H NMR spectra were recorded on a 400 MHz Bruker DPX-400 spectrometer. IR spectra were recorded on a Horiba FT-720 spectrometer or Shimadzu IR-460 spectrometer.

Results and discussion

Enzymatic oxidative polymerization of 4-hydroxyphenyl benzoate. The peroxidase-catalyzed polymerization of **1** was carried out with hydrogen peroxide as an oxidizing agent at room temperature for 3 h under air. The enzymes employed in this study were horseradish and soybean peroxidases (HRP and SBP), respectively. The

catalytic activity of SBP determined using guaiacol as a substrate is ca. quarter as large as that of HRP, then four times amount of SBP was employed. Both peroxidases were active for the oxidative polymerization of various phenol and aniline derivatives.[7,8,16]

In using a mixture of phosphate buffer (pH 7) and acetone (30:70 vol%) as a solvent, the powdery precipitates were formed during the polymerization, which was isolated by centrifugation. The yield of the polymer obtained by using SBP was larger than that from HRP (entries 1 and 2 in Table 1). A similar tendency was observed in the polymerization of bisphenol A and *m*-substituted phenols.[17,18] In the subsequent experiments, SBP is used as a catalyst.

Table 1. Enzymatic oxidative polymerization of 4-hydroxyphenyl benzoate (**1**). [a)]

Entry	Catalyst [b)]	Organic solvent	Buffer pH	Buffer content (vol%)	Yield (%)	Mn x 10^{-2} [c)]	Mw/Mn [c)]
1	HRP (1.0)	acetone	7	30	45	22	1.7
2	SBP (4.0)	acetone	7	30	75	24	1.8
3	SBP (4.0)	acetone	7	50	96	15	1.5
4	SBP (4.0)	1,4-dioxane	5	50	95	11	1.2
5	SBP (4.0)	1,4-dioxane	7	40	85	16	1.5
6	SBP (4.0)	1,4-dioxane	7	50	87	13	1.4
7	SBP (4.0)	1,4-dioxane	9	50	93	13	1.4
8	SBP (4.0)	THF	7	50	83	16	1.5

[a)] Polymerization of **1** (1.0 mmol) using hydrogen peroxide as an oxidizing agent in an aqueous organic solvent (10 mL) at room temperature for 3 h under air. [b)] In parenthesis, amount of enzyme in mg. [c)] Determined by SEC using THF eluent.

In the peroxidase-catalyzed polymerization of phenols in a mixture of buffer and organic solvent, the solvent composition, i.e., type of organic solvent, buffer pH and mixing ratio, strongly affected the yield, solubility, and molecular weight of the

polymer.[7,8,16] In this study, acetone, 1,4-dioxane, and tetrahydrofuran (THF) were used as organic solvent. In all cases examined, the polymer yield was high (more than 75 %). The polymerization in an equivolume mixture of buffer (pH 7) and acetone produced the polymer in a higher yield than that in the 30 % buffer content (entries 2 and 3). Effect of buffer pH was relatively small in the polymerization using an aqueous 1,4-dioxane as a solvent (entries 4, 6, and 7).

The solubility of the resulting polymer obtained in an equivolume mixture of buffer (pH 7) and 1,4-dioxane (entry 6) was examined (Table 2). The polymer was soluble in acetone, *N*,*N*-dimethylformamide (DMF), and dimethyl sulfoxide (DMSO), but insoluble in water, methanol, and hexane. The polymer molecular weight was estimated by size exclusion chromatographic (SEC) analysis using tetrahydrofuran (THF) eluent. The molecular weight was in the range of several thousands.

Table 2. Solubility of **2** and **3**. [a)]

Entry	Solvent	**2** (entry 6 in Table 1)	**3**
1	acetone	+	+
2	alkaline solution [b)]	-	+
3	chloroform	+	-
4	DMF	+	+
5	DMSO	+	+
6	hexane	-	-
7	methanol	-	+
8	THF	+	+
9	distilled water	-	±

[a)] +: Soluble; ±: partly soluble; -: insoluble. [b)] 1.0 N NaOH aqueous solution.

The polymer structure was analyzed by using ^{1}H NMR and IR spectroscopies. Figure 1(A) shows ^{1}H NMR spectrum of **1** in DMSO-d_6. Two doublet peaks at δ 6.8 and 7.1 are ascribed to the aromatic protons of phenolic moiety, and phenyl protons of the benzoate group were observed at δ 7.6, 7.7 and 8.1. A peak at δ 9.5 was due to the phenolic proton. The spectra pattern of **2** (Figure 1(B)) was similar to that of the monomer, although all the peaks, especially, peaks due to the aromatic protons derived from the phenol moiety, became broad. In the IR spectrum of the polymer (Figure 2(B)), observed were new peaks at 1264 and 1176 cm^{-1}, which are ascribed to the vibration of the C-O-C and C-OH linkages. These data show that the enzymatically obtained polymer from **1** is composed of a mixture of phenylene and oxyphenylene units.

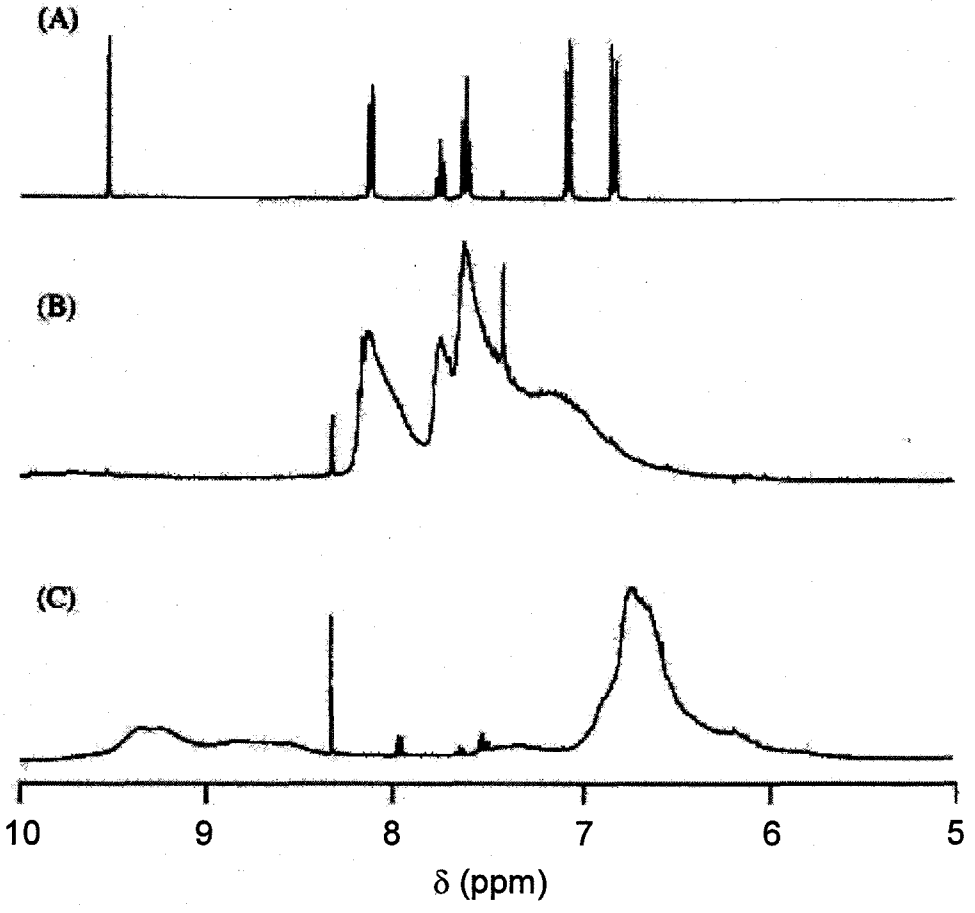


Figure 1. ^{1}H NMR spectra of (A) **1**, (B) **2**, and (C) **3**.

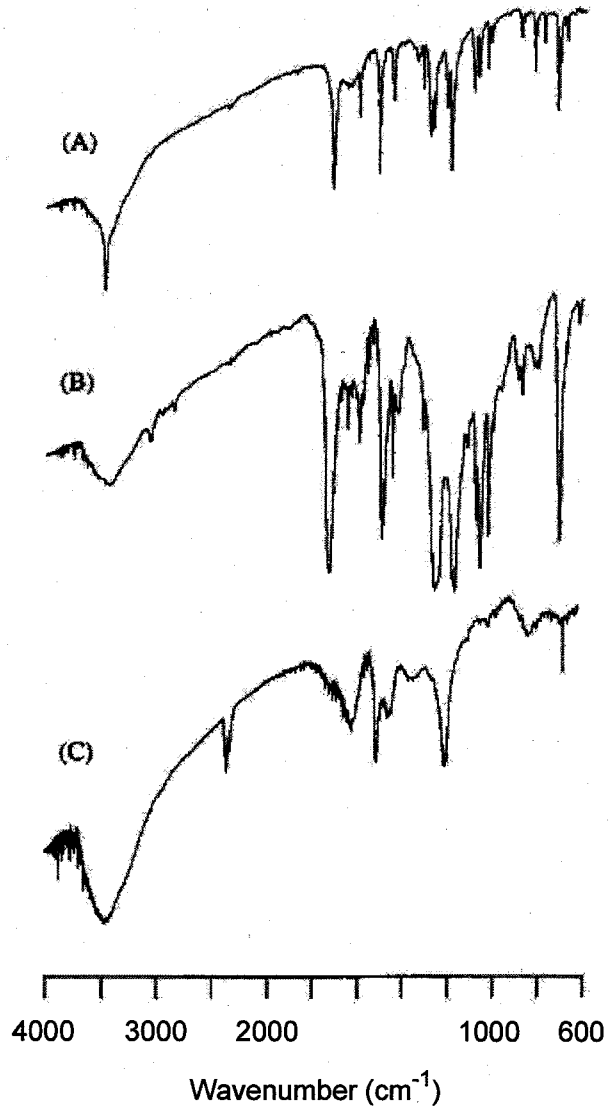


Figure 2. IR spectra of (A) **1**, (B) **2**, and (C) **3**.

The polymerization was carried out in a preparative scale (15 folds as described in experimental section) under the same conditions of entry 6 to give the polymer in 90 % yield, in which molecular weight and its index were 1500 and 1.5, respectively. The amount of the residual phenolic group of the polymer was determined by conventional titration methods.[18] The ratio of phenylene and oxyphenylene units was determined as 61:39.

Synthesis of poly(hydroquinone). The hydrolysis of **2** was carried out by using an excess of potassium hydroxide in a mixed solvent of THF and water (80:20 vol %). By the addition of potassium hydroxide at room temperature, the precipitates were immediately formed afterwards, the reaction mixture was heated at 60 °C for 24 h to complete the hydrolysis.

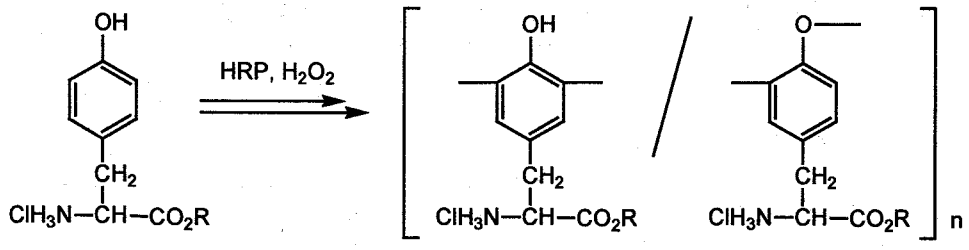
Figure 1(C) shows ^{1}H NMR spectrum of hydrolyzed polymer (**3**) in DMSO-d_6. The broad peaks due to the aromatic protons of the benzoate group at δ 7.3-8.2 completely disappeared. The peaks ascribed to the aromatic protons of the polymer backbone were somewhat shifted to the higher magnetic field. In the IR spectrum of **3** (Figure 2(C)), a characteristic peak at 1738 cm^{-1} ascribed to the carbonyl group of the benzoate shown in the IR spectra of polymer **2** was not observed. A broad strong peak at 1200 cm^{-1} due to the vibration of the C-O-C and C-OH linkages newly appeared and the peaks at 1264 and 1176 cm^{-1} shown in that of **2** disappeared. These data shows that the quantitative hydrolysis of the ester bond in **2** took place, yielding the poly(hydroquinone).

The solubility of the hydrolyzed product was different from that before the hydrolysis (Table 2). Polymer **3** was readily soluble in an alkaline aqueous solution and methanol, and insoluble in chloroform. On the other hand, the reverse tendency of the solubility was observed in **2**.

HRP-catalyzed polymerization of tyrosine ester hydrochlorides. At first, the HRP-catalyzed polymerization of L-tyrosine ethyl ester hydrochloride (**4a**) was carried out using hydrogen peroxide as an oxidizing agent in a Tris buffer (pH 7.6) at room temperature (Scheme 2). In the polymerization in 0.10 M Tris buffer, no polymeric precipitates were formed. The reaction mixture was poured into a large amount of acetone to give the powdery polymer in 19% yield. The molecular weight of the polymer, estimated by SEC with DMF containing 0.10 M LiCl as eluent, was 2400.

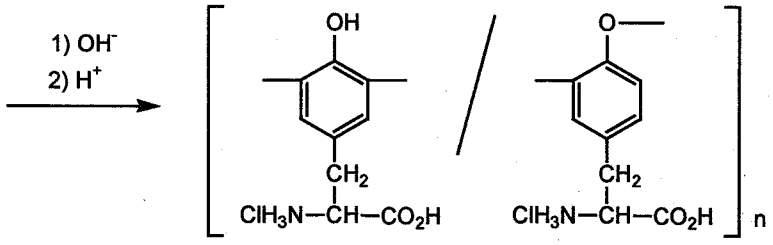
In distilled water, the formation of polymeric precipitates was not observed during the polymerization.

Scheme 2.



4 5

4a : L-Form, R = Et
4b : L-Form, R = Me
4c : D-Form, R = Me
4d : D,L-Form, R = Me



6

On the other hand, the powdery polymer was precipitated in high buffer concentration (1.0 M) during the reaction, which was collected by centrifugation after the reaction. This is because the solution became acidic due to the ammonium group in the case of dilute buffer concentration, which would affect enzyme activity and solubility of the resultant polymer. The resulting polymer was soluble in DMF, DMSO, methanol, and acidic and basic aqueous solutions but insoluble in distilled water, acetone, acetonitrile, and tetrahydrofuran. The polymer structure was estimated to be of a mixture of phenylene and oxyphenylene units by NMR and IR analysis.[18,19] Polymerization results are summarized in Table 3. The polymer yield strongly depended on the monomer concentration; there was a maximum point at the concentration of 160

mM (entry 3 in Table 3), indicating higher concentration of the monomer give good yield as long as the buffer capacity allows.

Table 3. HRP-catalyzed polymerization of tyrosine ester hydrochlorides (**4**). [a]

Entry	Monomer [b]	Yield (%)	Mn x 10^{-2} [c]	Mw/Mn [c]
1	**4a** (1.0)	35	15	1.8
2	**4a** (2.0)	74	17	2.5
3	**4a** (4.0)	82	19	3.6
4	**4a** (8.0)	24	22	1.7
5	**4b** (2.0)	64	40	3.4
6	**4c** (2.0)	64	40	3.1
7	**4d** (2.0)	63	25	3.4

[a] Polymerization of **4** (1.0 mmol) using HRP catalyst (10 mg) in 1.0 M Tris buffer (pH 7.6, 25 mL) at room temperature for 3 h under air. [b] In parenthesis, amount of monomer (mmol). [c] Determined by SEC using DMF containing 0.10 M LiCl eluent.

To examine the effect of the stereoisomer on the present polymerization, L-, D-, and D,L-tyrosine methyl ester hydrochlorides (**4b**, **4c**, and **4d**, respectively) were polymerized by HRP in the Tris buffer. The solubility of **4b** and **4c** toward the buffer was not high; thus, the low concentration of the monomer was used. The polymerization results of **4b** were very close to those of **4c**, suggesting that the stereoconfiguration of the tyrosine derivatives scarcely affected the present polymerization although it is reported that HRP showed stereospecificity with the oxidative reaction rate of

D-tyrosine being twice as fast as that of L-tyrosine.[20] The solubility of the polymer from **4b**, **4c**, and **4d** decreased, as compared with that from **4a**; the polymer was only soluble in DMF, DMSO, and acid and basic aqueous solutions.

Alkaline hydrolysis of poly(tyrosine ester) to a new class of poly(tyrosine). Poly(**4a**) (entry 3 in Table 3) was converted to a new class of poly(tyrosine) (**6**) by alkaline hydrolysis. The resulting product was only soluble in water. The molecular weight of **6** was 2300, estimated by SEC with water containing 0.10 M NaCl as eluent using poly(ethylene oxide) standard, whose value was relatively close to that of poly(**4a**). Figure 3 shows FT-IR spectra of poly(**4a**) and **6**. In the spectrum of poly(**4a**), there was a characteristic peak at 1730 cm^{-1} due to the C=O vibration of the ester moiety, which completely disappeared after the reaction. A new strong peak at 1635 cm^{-1} ascribed to the carboxylate salt was seen in the FT-IR spectrum of **6**. The ethyl ester group was not observed in the ^1H NMR measurement of **6** in D_2O. These data indicate that the ester moiety was hydrolyzed to form poly(tyrosine) having no peptide bond, whose structure was different from that of the commercially available poly(tyrosine). Thus, the present polymer is regarded as a new class of poly(amino acid).

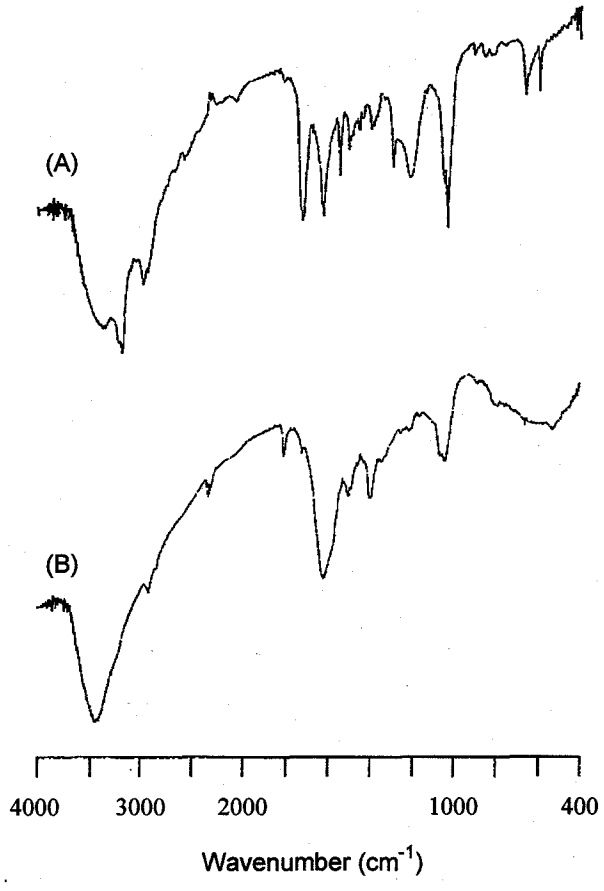


Figure 3. FT-IR spectra of (A) poly(**4a**) and (B) **6**.

Conclusion

4-Hydroxyphenyl benzoate (**1**) was oxidatively polymerized by the peroxidase catalyst to give a phenolic polymer **2** having the ester group in the side chain. The polymerization using soybean peroxidase in a mixed solvent of 1,4-dioxane and phosphate buffer (pH 7) afforded soluble polymer **2** in good yields. Polymer **2** was subjected to the alkaline hydrolysis, yielding a new type of hydroquinone polymer **3**; the structure of the polymer was different from that from hydroquinone or the hydroquinone derivative hitherto reported. Peroxidase-catalyzed oxidative polymerization of tyrosine ester hydrochlorides produced a new class of poly(amino acid)s. The stereoconfiguration of the monomer scarcely affected the polymerization behaviors. The alkaline hydrolysis of the resulting polymer afforded water-soluble poly(tyrosine), whose structure was different from that of polypeptides. Applications of the chemoenzymatically synthesized poly(hydroquinone) and poly(tyrosine) are expected to be developed for various purposes.

References

1) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.

2) Wang, P.; Dordick, J. S. *Macromolecules* **1998**, *31*, 941.

3) Novák, P.; Müller, K.; Santhanam, K. S. V.; Haas, O. *Chem. Rev.* **1997**, *97*, 207.

4) Foos, J. S.; Erker, S. M.; Rembetsy, L. M. *J. Electrochem. Soc.* **1986**, *133*, 839.

5) Wang, P.; Martin, B. D.; Parida, S.; Rethwisch, D. G.; Dordick, J. S. *J. Am. Chem. Soc.* **1995**, *117*, 12885.

6) Wang, P.; Amarasinghe, S.; Leddy, J.; Arnold, M.; Dordick, J. S. *Polymer* **1998**, *39*, 123.

7) Kobayashi, S.; Shoda, S.; Uyama, H. In *The Polymeric Materials Encyclopedia*; Salamone, J. C. Ed.; CRC Press: Boca Raton, 1996, p2102.

8) Kobayashi, S.; Shoda, S.; Uyama, H. In *Catalysis in Precision Polymerization*, Kobayashi S. Ed.; John Wiley & Sons: Chichester, 1997; Chapter 8.

9) In *Biopolymers from Renewable Resources*; Kaplan, D. L. Ed.; Springer: Berlin, 1998.

10) Gross, R. A.; Scholz, C., Eds. *ACS Symp. Ser.* **2001**, *No. 786.*

11) Rathore, O.; Winningham, M. J.; Sogah, D. Y. *J. Polym. Sci., Polym. Chem. Ed.* **2000**, *38*, 352.

12) Sanda, F.; Endo, T. *Macromol. Chem. Phys.* **1999**, *200*, 2651.

13) Malencik, D. A.; Anderson, S. R. *Biochemistry* **1996**, *35*, 4375.

14) Gmeiner, P.; Feldman, P. L.; Chu-Moyer, M. Y.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 3068.

15) Witt, O. N.; Johnson, E. S. *Chem. Ber.* **1893**, *26*, 1909.

16) Gross, R. A.; Kaplan, D. L.; Swift, G. (Ed.), *ACS Symp. Ser.* **1998**, *684*.

17) Kobayashi, S.; Uyama, H.; Ushiwata, T.; Uchiyama, T.; Sugihara, J.; Kurioka, H. *Macromol. Chem. Phys.* **1998**, *199*, 777.

18) Tonami, H.; Uyama, H.; Kobayashi, S.; Kubota, M. *Macromol. Chem. Phys.* **1999**, *200*, 2365.

19) Uyama, H.; Maruichi, N.; Tonami, H.; Kobayashi, S. Biomacromolecules **2002**, *3*, 187.

20) Bayse, G. S.; Michaels, A. W.; Morrison, M. *Biochim. Biophys. Acta* **1972**, *284*, 34.

Chapter 4

Enzymatic Polymerization of *m*-Substituted Phenols in the Presence of Heptakis(2,6-di-*O*-methyl)-β-cyclodextrin in Water

Introduction

There has been much interest in supramolecular chemistry using cyclodextrins (CDs) as host molecules. CDs are cyclic oligoamyloses providing hydrophobic cavity ranging from ca. 6 Å to 10 Å and were found to form inclusion complexes with various polymers.[1-3]

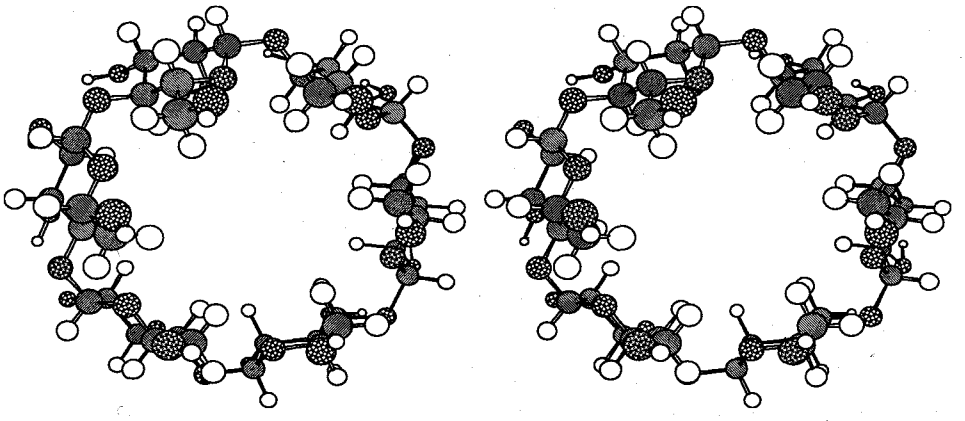


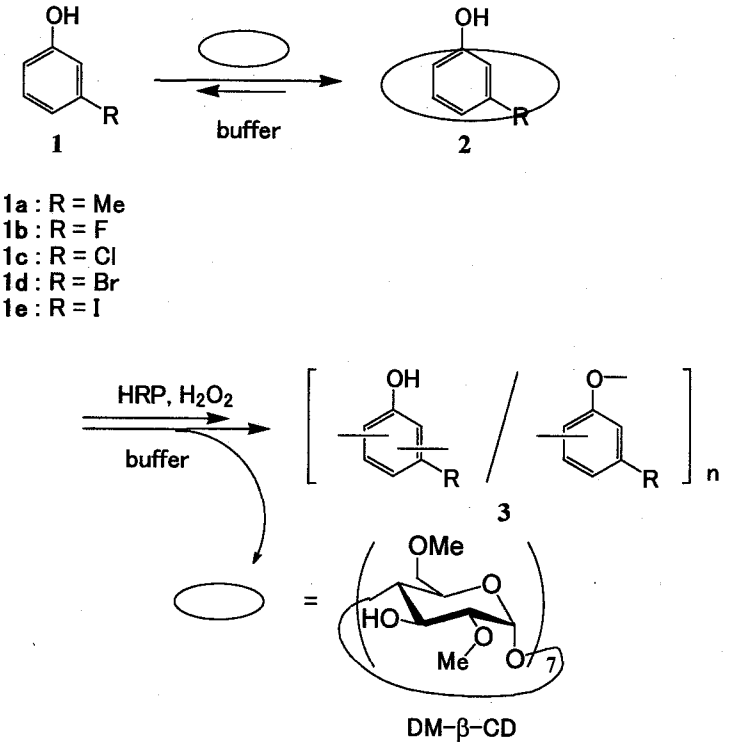
Figure 1. A stereo drawing of α-CD from 6-OH direction.

CDs also form water-soluble inclusion complexes with many organic compounds showing low solubility toward water. Recently, Ritter and co-workers have reported that water-soluble complexes are formed from hydrophobic monomers such as styrene or (meth)acrylates with methylated β-CD in water and the complexes were subjected to free-radical polymerizations and copolymerizations, yielding water-insoluble vinyl polymers in high yields.[4,5] During the polymerization, the CD

generally slips off from the guest component and remains in the aqueous phase owing to its high water solubility, then the resulting polymer precipitates. Furthermore, the degree of the polymerization could be controlled by the addition of a water-soluble chain transfer agent (*N*-acetyl-L-cystein).[6] Under the atom-transfer conditions, the polymerization of the CD-complexed vinyl monomers showed a living character.[7]

In the enzymatic polymerization of unsubstituted phenol, use of an aqueous methanol solvent afforded the soluble polyphenol showing high thermal stability.[8,9] *m*-Substituted phenols were enzymatically polymerized in an aqueous methanol to produce the polymer readily soluble in polar solvents such as acetone and methanol.[10] For the efficient enzymatic production of the soluble phenolic polymers, organic solvents are often required as cosolvents except highly hydrophilic phenolic monomers.[8-18] The polymerization in water often gave the insoluble polymer in low yields.[9] Very recently, the polymerization in the presence of CD derivatives has been expanded to the HRP-catalyzed oxidative coupling; water-soluble complexes of *p*-substituted hydrophobic phenols and heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) were formed in water and oxidatively polymerized by HRP catalyst.[19] This chapter deals with HRP-catalyzed polymerization of *m*-substituted phenols (**1**) in a buffer in the presence of DM-β-CD (Scheme 1), in which the soluble polymer was obtained in high yields without organic solvents.

Scheme 1.



Experimental section

Materials. *m*-Substituted phenols and DM-β-CD were commercially available and used as received. Horseradish peroxidase (HRP) (EC 1.11.1.7, 100U/mg) was purchased from Wako Pure Chemical Co. and used without further purification.

Enzymatic reaction. The following is a typical procedure for the polymerization (entry 1). Under air, *m*-substituted phenol (2.0 mmol) and HRP (10 mg) in 0.1 M phosphate buffer (pH 7.0) (10 mL) were placed in a 50 mL flask. DM-β-CD (2.2 mmol) was added to the solution and vigorously stirred until the solution became clear. Hydrogen peroxide (5.0 % aq. solution, 1.36 mL, 2.0 mmol) was added dropwise to the mixture for 2 h at room temperature under air. After the subsequent stirring of the

reaction mixture for an hour, the resulting precipitates were collected by centrifugation. The precipitates were dissolved in a small amount of tetrahydrofuran and reprecipitated by pouring into a large amount of water, followed by washing with water repeatedly to remove DM-β-CD completely. The purified precipitates were dried in vacuo to give 0.22 g of the polymer (yield 100 %).

Measurement. SEC analysis was carried out using a TOSOH SC8010 apparatus with a refractive index (RI) detector at 40 °C under the following conditions: TSKgel G3000H_{HR} column and THF eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. ^{1}H NMR and 2D-NOESY spectra were recorded on a 400 MHz Bruker DPX-400 spectrometer.

Results and discussion

Formation of inclusion complexes. Here *m*-substituted phenols, *m*-cresol (**1a**), *m*-fluorophenol (**1b**), *m*-chlorophenol (**1c**), *m*-bromophenol (**1d**), and *m*-iodophenol (**1e**) were used. Even in case of phenols showing low solubility toward water (**1a**, **1c**, **1d**, and **1e**), water-soluble inclusion complexes (**2**) consisting of DM-β-CD and **1** were readily formed by stirring them at room temperature; the heterogeneous aqueous solution of **1** became a clear homogeneous one after the addition of a slight excess of DM-β-CD.

The formation of **2** was confirmed by NMR spectroscopies in D_2O. ^1H NMR spectroscopy has been often used to confirm complexation by the change of chemical shifts.[19] In the ^1H NMR spectrum of an equimolecular mixture of **1a** and DM-β-CD, a distinct change was observed with comparison of that of **1a** or DM-β-CD (Figure 2). Methyl protons of **1a** were shifted from δ 2.21 to a lower magnetic field of δ 2.25, on complexation; on the other hand, a reverse tendency was seen for aromatic protons of **1a** and CD protons. Similar behaviors were also observed in spectra of *m*-halogenated phenols (**1b-1d**). More clear evidence of the complexation was obtained by two-dimensional nuclear Overhauser effect spectroscopy (2D-NOESY) (Figure 3),

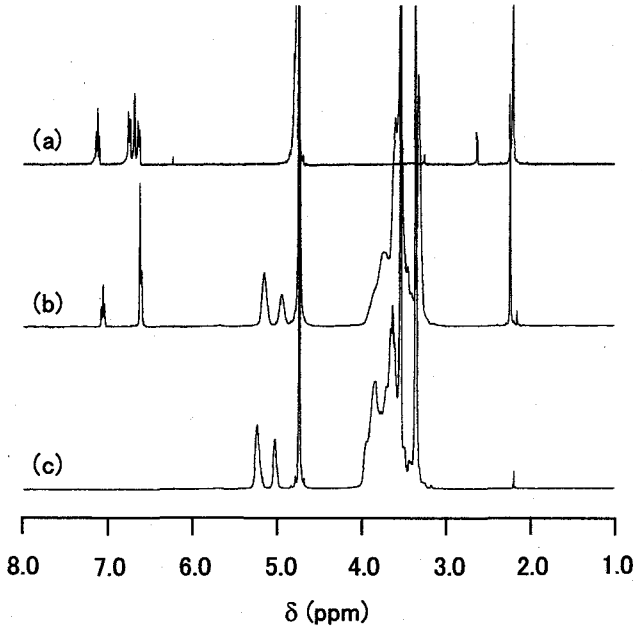


Figure 2. ^1H NMR spectra of (a) *m*-cresol, (b) an equimolecular mixture of *m*-cresol and DM-β-CD and (c) DM-β-CD in D_2O.

which has been extensively used for structural characterization, especially information of proton vicinity.[21,22] 2D-NOESY NMR spectrum of an equimolecular mixture of **1a** and DM-β-CD in D_2O showed intermolecular cross peaks between methyl and aromatic protons of **1a** and DM-β-CD, as pointed out by the circles in Figure 3. These data clearly indicate the proximity of **1a** and the inner protons of DM-β-CD by the inclusion complex formation.

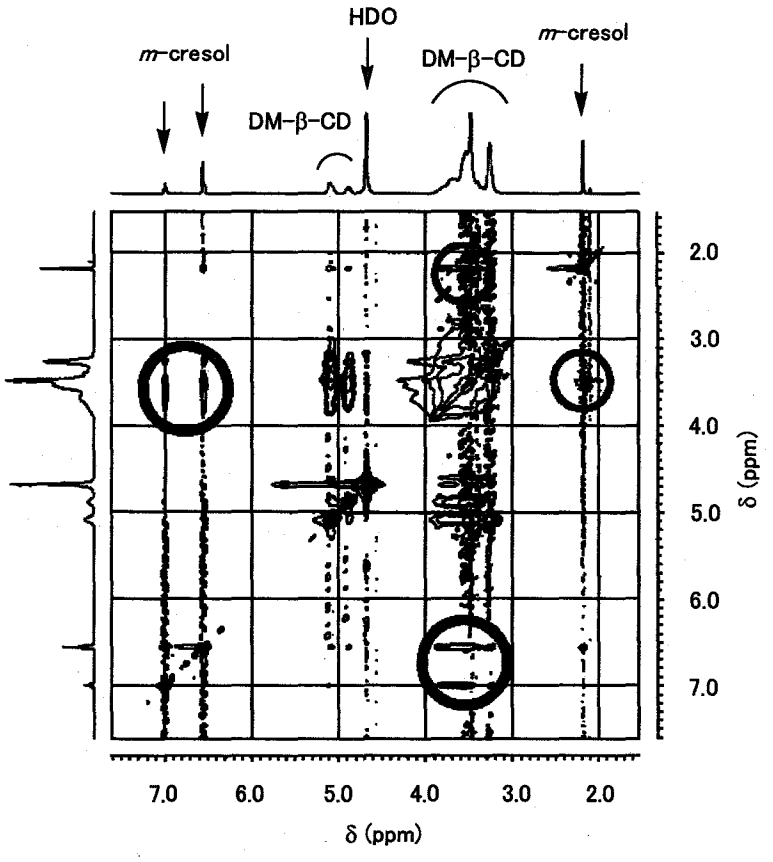


Figure 3. 2D-NOESY spectrum of an equimolecular mixture of *m*-cresol and DM-β-CD in D_2O.

Association constant (K_a) for **2** was measured according to Benesi-Hildebrand method.[20,23]

$$1 / \Delta\delta = \{1 / (K_a \times \Delta\delta_C)\}\ 1 / [\beta\text{-CD}]_0 + 1 / \Delta\delta_C \qquad (1)$$

where $\Delta\delta$ is the observed change in chemical shift, $\Delta\delta_C$ is the difference in chemical shift between the free and complexed states, $[\beta\text{-CD}]_0$ is the total β-CD concentration and K_a is the association constant obtained from the ratio of intercept to slope from a plot $1 / \Delta\delta$ vs. $1 / [\beta\text{-CD}]_0$. Figure 4 shows effects of the concentration of DM-β-CD on the ^1H or ^{19}F NMR chemical shift of monomers. For all the monomers except **1e**, there was a linear relationship between reciprocals of them, and K_a is calculated from the ratio of the intercept to slope. K_a values were higher than 100 M^{-1} (Table 1), which was enough large to solubilize the monomer in the present concentration.

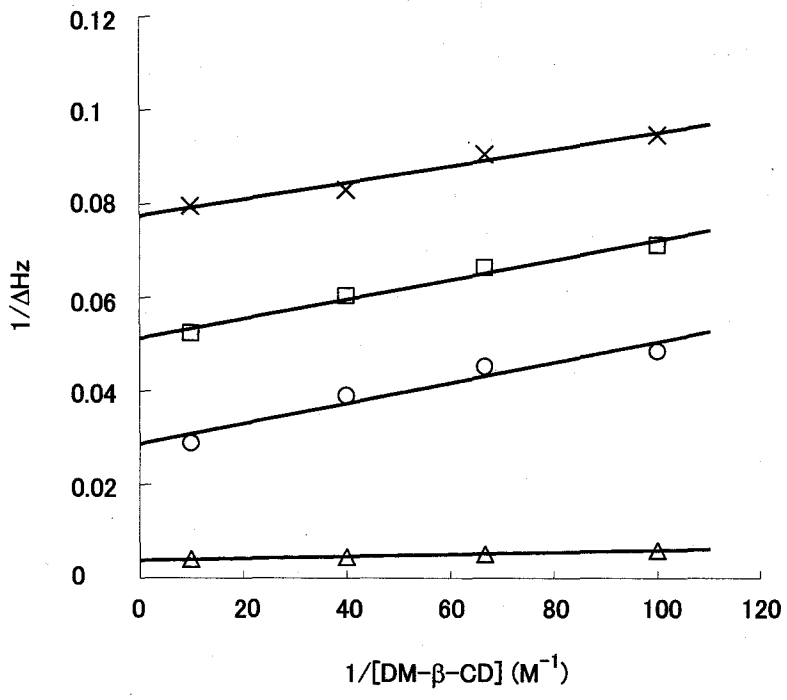


Figure 4. Determination of the association constants of inclusion complexes (**2**) according to the Benesi-Hildebrand plots: (o) *m*-cresol; (Δ) *m*-fluorophenol; (□) *m*-chlorophenol; (x) *m*-bromophenol.

Molecular mechanics calculations were performed using MM2. The reference β-CD structure was obtained by minimizing a crystallographic geometry. After adding *m*-cresol as a guest molecule at the center of CD cavity and methyl groups at each 2- and 6- OH group, the system is minimized. The resulting geometry is shown in Figure 5. Similar results were obtained in cases of the other halogenated phenols as well as *m*-cresol.

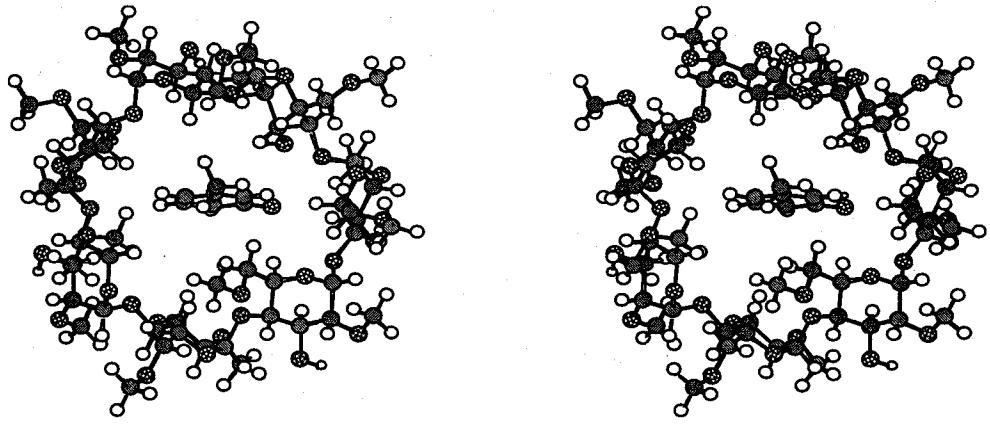


Figure 5. Optimized structure of the *m*-cresol complex with DM-β-CD (stereo view).

Enzymatic reaction. After the complexation, the oxidative polymerization was carried out by HRP catalyst. Polymerization results are summarized in Table 1, in which results in the absence of DM-β-CD are shown for comparison. During the polymerization, polymeric precipitates were formed, which were collected by centrifugation. Residual DM-β-CD was removed by reprecipitation and washing with water. The presence of DM-β-CD efficiently produced polymer (**3**) soluble in polar organic solvents such as acetone, N,N-dimethylformamide (DMF), dimethyl sulfoxide,

Table 1. HRP-catalyzed polymerization of *m*-substituted phenols (**1**) in water in the presence of DM-β-CD. [a)]

Entry	**1** Substituent R	K_a [b)] (M^{-1})	[DM-β-CD] (mmol)	Yield (%)	Mn x 10^{-2} [c)]	Mw/Mn [c)]
1	Me	132	2.2	100 [d)]	10	1.7
2	Me		0	22 [d)]	--- [e)]	--- [e)]
3	F	181	2.2	77 [d)]	12	1.7
4	F		0	23 [d)]	--- [e)]	--- [e)]
5	Cl	244	2.2	88 [d)]	9.3	1.3
6	Cl		0	0 [f)]	---	---
7	Br	438	2.2	87 [d)]	8.1	1.4
8	Br		0	0 [f)]	---	---
9	I	--- [g)]	2.2	80 [d)]	7.2	1.3
10	I		0	0 [f)]	---	---

[a)] Polymerization of **1** (2.0 mmol) using HRP catalyst (10 mg) in pH 7.0 phosphate buffer (10 mL) at room temperature for 3 h. [b)] Determined by NMR. [c)] Determined by SEC using THF eluent. [d)] Water-insoluble part. [e)] Insoluble in THF. [f)] Unreacted monomer was recovered. [g)] Not determined due to small peak shift.

and tetrahydrofuran (THF). On the other hand, the polymer yield dramatically decreased in the absence of DM-β-CD probably due to low conversion of the phenolic monomers.[9] Furthermore, the polymers obtained in the absence of DM-β-CD showed very low solubility toward any solvents. These results indicate that the complexation of phenols by DM-β-CD plays a very important role for the polymerization of hydrophobic phenols in water. It is interesting that only phenols free from DM-β-CD are likely to be polymerized by HRP because these inclusion complexes looks too bulky to be recognized to the binding site of HRP, nevertheless, DM-β-CD provided efficient reaction system by homogenizing solution and releasing monomer gradually and slowly. NMR and IR spectra of the present polymer were similar to those previously obtained without DM-β-CD in aqueous organic solvents.[10,24] Therefore they would be consisting of biphenyl linkages and phenoxy ether linkages like previous reports.

Conclusion

m-Substituted phenols (**1**) were successfully polymerized by HRP catalyst in water in the presence of DM-β-CD, yielding the soluble polyphenols in high yields. NMR analysis exhibited the complexation of **1** and DM-β-CD. Monomers **1a-1d** possessed the large association constant of the complexation. The present study clearly shows that commercially available DM-β-CD is found to be a very useful additive for efficient production of the polymer without use of organic solvents.

References

1) Harada, A. *Adv. Polym. Sci.* **1997**, *133*, 141.

2) Nepogodiev, S. A.; Stoddard, J. F. *Chem. Rev.* **1998**, *98*, 1959.

3) Harada, A.; Li, J.; Kamachi, M. *Nature* **1992**, *356*, 325.

4) Storsberg, J.; Ritter, H. *Macromol. Rapid Commun.* **2000**, *21*, 236.

5) Casper, P.; Glöckner, P.; Ritter, H. *Macromolecules* **2000**, *33*, 4361.

6) Glöckner, P.; Metz, N.; Ritter, H. *Macromolecules* **2000**, *33*, 4288.

7) Storsberg, J.; Hartenstein, M.; A. Müller, H. E.; Ritter, H. *Macromol. Rapid Commun.* **2000**, *21*, 1342.

8) Oguchi, T.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **1999**, *20*, 401.

9) Oguchi, T.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Bull. Chem. Soc. Jpn.* **2000**, *73*, 1389.

10) Tonami, H.; Uyama, H.; Kobayashi, S; Kubota, M. *Macromol. Chem. Phys.* **1999**, *200*, 2365.

11) Kobayashi, S.; Shoda, S.; Uyama, H. *Adv. Polym. Sci.* **1995**, *121*, 1.

12) Kobayashi, S.; Shoda, S.; Uyama, H. In *The Polymeric Materials Encyclopedia*; Salamone, J. C. Ed.; CRC Press: Boca Raton, 1996; p 2102.

13) Kobayashi, S.; Shoda, S.; Uyama, H. In *Catalysis in Precision Polymerization;* Kobayashi, S. Ed.; John Wiley & Sons: Chichester, 1997, Chapt. 8.

14) Ritter, H. In *Desk Reference of Functional Polymers, Syntheses and Applications*; Arshady, R. Ed.; American Chemical Society: Washington, 1997; p 103.

15) Gross, R. A.; Kaplan, D. L.; Swift, G. (Eds.), *ACS Symp. Ser.* **684** (1998).

16) Kobayashi, S. *J. Polym. Sci., Polym. Chem. Ed.* **1999**, *37*, 3041.

17) Kobayashi, S.; Uyama, H.; Ohmae, M. *Bull. Chem. Soc. Jpn.* **2001**, *74*, 613.

18) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793.

19) Reihmann, M. H.; Ritter, H. *Macromol. Chem. Phys.* **2000**, *201*, 798.

20) Divakar, S.; Maheswaran, M. M. *J. Inclusion Phenom. Mol. Recognit. Chem.*

1997, *27*, 113.

21) Bodenhausen, G.; Kogler, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370.

22) Kelts, L. W.; Landry, C. J. T.; Teegarden, D. M. *Macromolecules* **1993**, *26*, 2941.

23) Velty, R. A.; Rinaudo, M. *Macromolecules* **2001**, *34*, 3574.

24) Ikeda, R.; Maruichi, N.; Tonami, H.; Tanaka, H.; Uyama, H.; Kobayashi, S. *J. Macromol. Sci.-Pure Appl. Chem.* **2000**, *A37*, 983.

Chapter 5

Iron Salen-Catalyzed Oxidative Polymerization of Phenol Derivatives

Introduction

Oxidative polymerizations have afforded various functional polymers. Typical examples are polyaniline, polypyrrole, and polythiophene showing high conductivity.[1-3] These polymers are synthesized by electrolysis or chemical oxidation processes. Another example is poly(1,4-oxyphenylene) (poly(phenylene oxide), PPO), which was first synthesized from 2,6-dimethylphenol by using a copper/amine catalyst.[4,5] PPO is widely used as high-performance engineering plastics in industrial fields, since the polymer has excellent chemical and physical properties, e.g., a high glass transition temperature (ca. 210 °C) and mechanically tough property.[6]

Complexes of a metal with *N,N'*-ethylenebis(salicylideneamine) (salen) or its derivatives have been extensively studied as oxidation catalysts. In particular, Mn(III) and Co(II)-salens showed excellent catalytic ability toward enantioselective epoxidation of olefins and versatile synthesis of alkoxyamines.[7-10] Immobilization of these complexes onto the synthetic polymers was performed to develop a polymeric catalyst for the highly selective reactions.[11,12]

Here the author picked up a five-coordinated (μ-oxo)diiron(III) complex,[15] as a mimic of peroxidases. The crystal structures of a wide variety of these complexes are available (Figure 1).[13-17] The relationship between the structure and the function has been under discussion to get more insight about the active centers of the proteins.[18-22] In most of oxidative polymerizations of phenol derivatives except those using peroxidase catalyst, oxygen molecules and metal oxides were used as oxidizing agents; so far, there

were few reports on the oxidative polymerization using hydrogen peroxide.[23] This chapter deals with the oxidative polymerization of phenol derivatives, especially focused on 2,6-disubstituted phenol derivatives (**1**), catalyzed by Fe(III)-salen complex (**2**) using hydrogen peroxide as an oxidizing agent (Scheme 1). This is the first example that a metal-salen complex showed high catalytic activity toward the oxidative polymerizations.

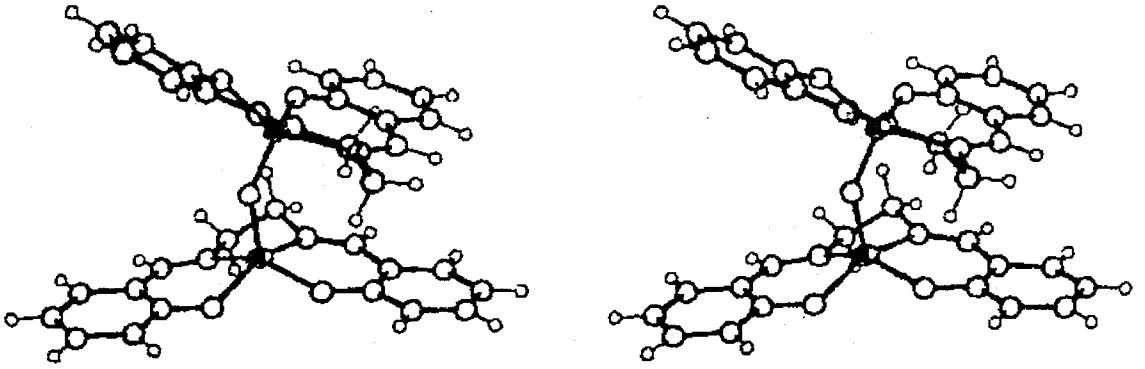
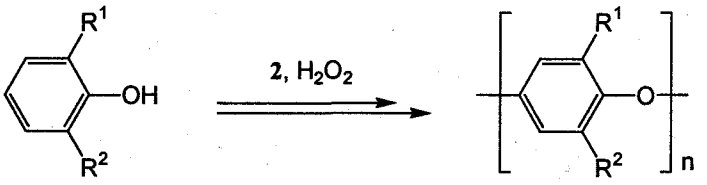


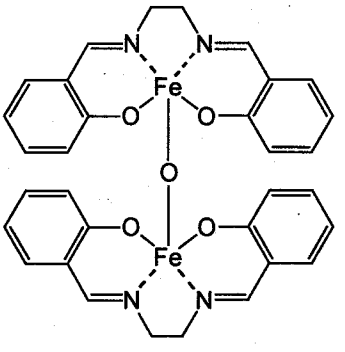
Figure 1. Stereoscopic illustration of the molecular geometry of $[Fe(salen)_2]O$.

Scheme 1.



1

1a : $R^1 = R^2 = CH_3$
1b : $R^1 = CH_3$, $R^2 = CH_2CH{=}CH_2$
1c : $R^1 = R^2 = i\text{-}C_3H_7$
1d : $R^1 = R^2 = OCH_3$
1e : $R^1 = R^2 = Ph$



2

Experimental section

Materials. Fe-salen complex (**2**) was synthesized according to the literature.[24] Other reagents and solvents were commercially available and used as received.

Oxidative polymerization of phenol derivatives. A typical run was as follows (entry 4 in Table 1). 2,6-Dimethylphenol (0.61 g, 5.0 mmol), **2** (3.3 mg, 5.0 μmol) and pyridine (0.10 mL) in 10 mL of 1,4-dioxane were placed in a 50 mL of flask. 5 % Hydrogen peroxide (3.4 mL, 5.0 mmol) was added dropwise for 1 h at room temperature. The mixture was stirred under air. After 3 h, the reaction mixture was poured into a large amount of methanol. The precipitates were separated by centrifugation and washed with methanol, following by drying in vacuo to give the polymer (0.56 g, 91 % yield).

Measurements. Size exclusion chromatographic (SEC) analysis was carried out using a Tosoh SC8010 apparatus with a refractive index (RI) detector under the following conditions: TSKgel G3000H_{HR} column and tetrahydrofuran (THF) eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. NMR spectra were recorded on a 270 MHz JEOL JNM-EX270J or a 400MHz Bruker DPX-400 spectrometer. IR spectra were recorded on a Horiba FT720 spectrometer. DSC measurement was made at a 10 °C/min heating rate under nitrogen using a Seiko SSC/5200 differential scanning calorimeter calibrated with an indium reference standard. TG analysis was performed using a Seiko SSC/5200 apparatus for thermogravimetry / differential thermal analysis at a heating rate of 10 °C/min in a gas flow rate of 300 mL/min.

Results and discussion

Oxidative Polymerization of 2,6-Dimethylphenol Catalyzed by Fe(III)-Salen Complex. The oxidative polymerization of 2,6-dimethylphenol (**1a**) was performed by using Fe(III)-salen (**2**) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, at room temperature under air. As a polymerization solvent, 1,4-dioxane was used since it shows high solubility toward PPO and is miscible with aqueous hydrogen peroxide solution. Hydrogen peroxide was added dropwise to the reaction mixture for 1 h. By the addition of hydrogen peroxide, the reaction solution turned dark-red, afterwards the precipitates were formed. After 3 h, the products were isolated by pouring the reaction mixture into a large amount of methanol (yield 78 %). In the chart of SEC, two peaks were observed and their peak areas were almost the same. The number-average molecular weight and its index of the peak in lower elution volume were 1.6×10^4 and 1.5, respectively. ^{1}H NMR chart of the product shows two singlet peaks at δ 6.6 and 7.7 in the aromatic region (Figure 2), which were ascribed to methyl protons of poly(2,6-dimethyl-1,4-oxyphenylene) and a dimer of **1a**, 3,5,3',5'-tetramethyl-4,4'-diphenoquinone (DPQ), respectively.[25] The ratio of integrated areas of these peaks was 52:48, which was very close to that determined by SEC. Thus, the peak of higher elution volume in the SEC chart was ascribed to DPQ. The DPQ formation is explained by the para-para coupling of radical species from **1a**.

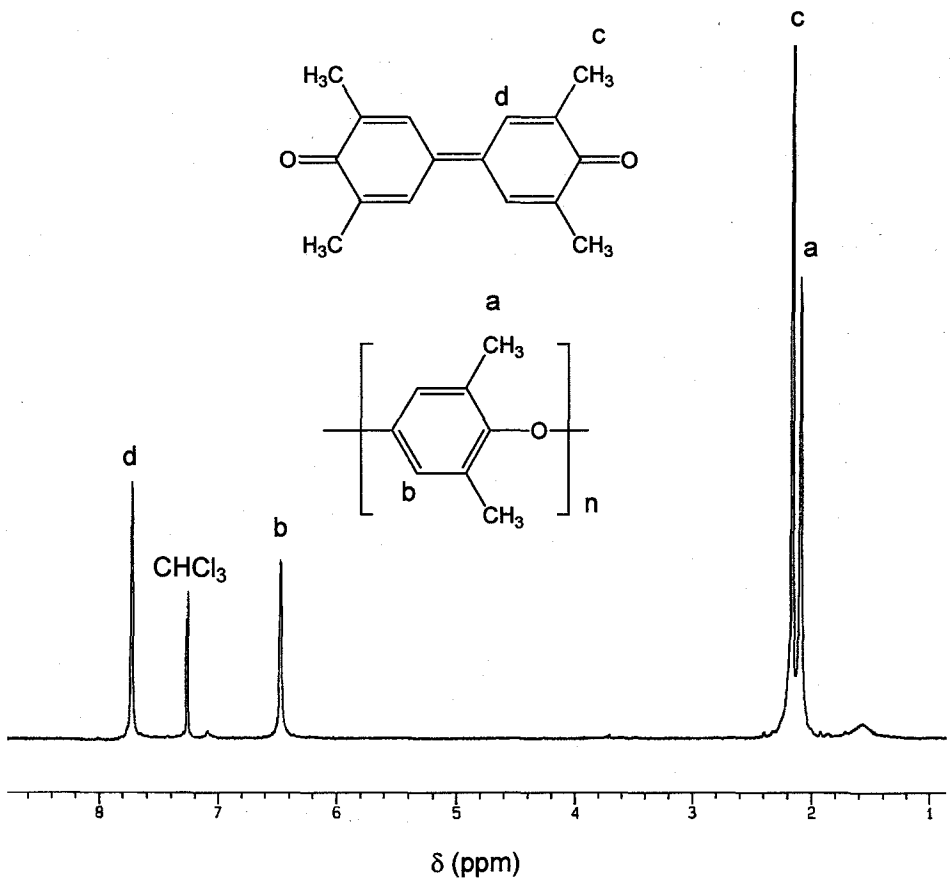


Figure 2. ^{1}H NMR spectrum of the product (entry 1 in Table 1).

In the oxidative polymerization of **1a** using a copper catalyst, the addition of amine suppressed the DPQ formation, yielding PPO.[26,27] Here, the polymerization of **1a** by using **2** as catalyst was performed in the presence of pyridine with different amount (Table 1). A small amount of pyridine was effective for the suppression of the DPQ formation (entry 2). As the added amount of pyridine, the content of the polymer in the methanol-insoluble part increased. DPQ was not formed in using 1.0 mL of pyridine (entry 5). These results indicate that the addition of pyridine effectively suppressed the DPQ formation. In the subsequent experiments, the amount of amine is fixed at 0.1 mL. The molecular weight of the polymer scarcely changed by the amount of pyridine.

Table 1. Effect of Pyridine Amount in the Oxidative Polymerization of 2,6-Dimethylphenol (**1a**) Catalyzed by Fe(III)-Salen Complex (**2**). [a)]

Entry	Amount of Pyridine (mL)	Yield [b)] (%)	Polymer Content [c)] (%)	Mn x 10^{-3} [d)]	Mw/Mn [d)]
1	0	78	50	16	1.5
2	0.001	100	93	15	1.6
3	0.01	88	97	16	1.5
4	0.1	91	99	11	1.4
5	1.0	89	100	12	1.4

[a)] Polymerization of **1a** (5.0 mmol) using **2** (5.0 μmol) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, in the presence of pyridine in 1,4-dioxane (10 mL) at room temperature for 3 h under air. [b)] Methanol-insoluble part. [c)] Polymer ratio in methanol-insoluble part, determined by SEC. [d)] Determined by SEC.

The oxidative polymerization of **1a** catalyzed by a copper/amine is known to involve several side reactions, resulting in Mannish-base- and DPQ-incorporations into the polymer.[28] The polymer structure was confirmed by using NMR spectroscopy. Before the measurement, the polymer was further purified by washing with acetone for the complete removal of DPQ. In the ^{1}H NMR spectrum (Figure 3), there are two main peaks at δ 2.1 and 6.6, which are due to the methyl and aromatic protons of 2,6-dimethyl-1,4-oxyphenylnene unit.[28,29] Beside these peaks, a multiplet peak at δ 7.1 ascribed to the terminal phenyl protons was observed. No additional peaks were detected in the ^{1}H NMR spectrum. These data indicate that the present polymer was composed of exclusively 1,4-oxyphenylene unit. ^{13}C NMR analysis supports the polymer structure.

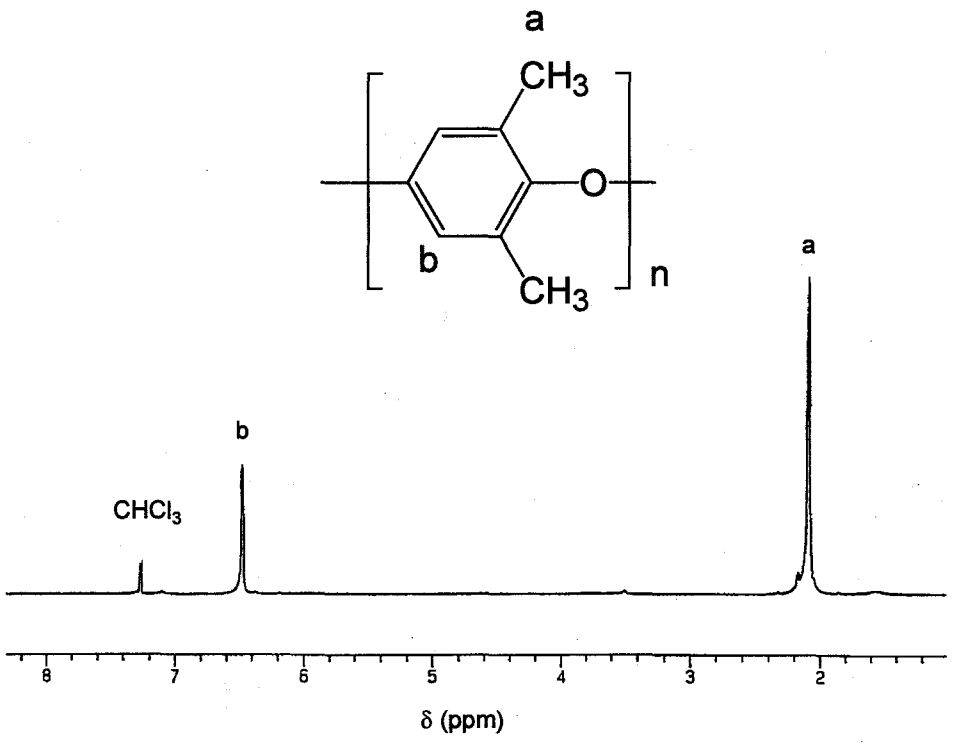


Figure 3. ^{1}H NMR spectrum of PPO obtained by the iron salen catalyst.

The effect of amine structure has been examined by using 2,6-lutidine, *N,N,N',N'*-tetraethylethylenediamine (TEED) and triethylamine (Table 2). The yield of the methanol-insoluble part and the molecular weight of the polymer were not so different with those in using pyridine. In all cases, the polymer content in the methanol-insoluble part was more than 96 %. These data suggest that the polymerization behavior was not much affected by the amine structure.

Table 2. Effect of Amine Structure in the Oxidative Polymerization of 2,6-Dimethylphenol (**1a**) Catalyzed by Fe(III)-Salen Complex (**2**) [a)]

Entry	Amine (mL)	Yield [b)] (%)	Polymer Content [c)] (%)	Mn x 10^{-3} [d)]	Mw/Mn [d)]
1	2,6-Lutidine	91	96	14	1.6
2	Pyridine	91	99	11	1.4
3	TEED [e)]	91	100	9.4	1.3
4	Triethylamine	91	100	9.1	1.3

[a)] Polymerization of **1a** (5.0 mmol) using **2** (5.0 μmol) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, in the presence of amine (0.1 mL) in 1,4-dioxane (10 mL) at room temperature for 3 h under air. [b)] Methanol-insoluble part. [c)] Polymer ratio in methanol-insoluble part, determined by SEC. [d)] Determined by SEC.
[e)] N,N,N',N'-tetraethylethylenediamine.

The polymerization has been performed in various organic solvents in the presence of pyridine (Table 3). In good solvents for PPO, i.e., 1,4-dioxane, THF, and dimethoxyethane, the polymer with molecular weight of more than $1x10^4$ was obtained in high yields (entries 2, 3, and 6). In these solvents, the polymer content in the methanol-insoluble part was very high ($\geq$ 98%). The highest molecular weight was achieved by using dimethoxyethane as a solvent (entry 2). Acetonitrile, *N,N*-dimethylformamide (DMF), and 2-propanol afforded the oligomer (entries 1, 4, and 5). This is probably due to the low solubility of PPO toward these solvents. ^{1}H NMR analysis showed that the DPQ content in the product was low (less than 10 %).

Table 3. Effect of Organic Solvent in the Oxidative Polymerization of 2,6-Dimethylphenol (**1a**) Catalyzed by Fe(III)-Salen Complex (**2**) [a)]

Entry	Amine (mL)	Yield [b)] (%)	Polymer Content [c)] (%)	Mn x 10^{-3} [d)]	Mw/Mn [d)]
1	Acetonitrile	91	91 [e)]	2.3	1.6
2	Dimethoxyethane	91	99	19	1.4
3	1,4-Dioxane	91	99	11	1.4
4	DMF	98	93 [e)]	1.9	1.4
5	2-Propanol	70	92 [e)]	2.2	1.4
6	THF	91	98	16	1.4

[a)] Polymerization of **1a** (5.0 mmol) using **2** (5.0 μmol) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, in the presence of pyridine (0.1 mL) in organic solvent (10 mL) at room temperature for 3 h under air. [b)] Methanol-insoluble part.
[c)] Polymer ratio in methanol-insoluble part, determined by SEC. [d)] Determined by SEC.
[e)] Determined by ^{1}H NMR.

The synthesis of phenylene oxide oligomer from **1a** was reported: peroxidase catalyst in an aqueous organic solvent[29] and copper/amine catalyst in a mixture of good and poor solvents for PPO.[28] Next, a mixed solvent of 1,4-dioxane and water was used in order to control the molecular weight (Table 4). By the addition of water, the molecular weight dramatically decreased. The decrease of the polymer yield was observed in the aqueous 1,4-dioxane, probably due to the loss of the oligomer during the purification procedure.

Table 4. Effect of Water Content in the Oxidative Polymerization of 2,6-Dimethylphenol (**1a**) Catalyzed by Fe(III)-Salen Complex (**2**) [a)]

Entry	Water Content (mL)	Yield [b)] (%)	Mn x 10^{-3} [c)]	Mw/Mn [c)]
1	0	91	11.0	1.4
2	20	74	3.8	1.6
3	40	56	2.3	1.5
4	60	56	1.5	2.1
5	80	50	1.6	2.0

[a)] Polymerization of **1a** (5.0 mmol) using **2** (5.0 μmol) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, in the presence of pyridine (0.1 mL) in a mixture of 1,4-dioxane and water (10 mL) at room temperature for 3 h under air. [b)] Methanol-insoluble part. [c)] Determined by SEC.

In this study, a very small amount of the catalyst (0.10 mol% for the monomer) afforded the polymer in high yields, showing the very efficient catalysis of **2**. The catalyst amount was decreased (0.01 mol% for **1a**) in the polymerization using 1,4-dioxane as a solvent in the presence of 0.001 mL of pyridine, resulting in the very low yield of the polymer (5 %). In the above experiments, hydrogen peroxide was added dropwise for 1 h. Next, hydrogen peroxide was added all at once in the monomer solution to give the polymer with molecular weight of 2500 in 15 % yield, suggesting that the dropwise addition of hydrogen peroxide was required for the efficient synthesis of PPO.

Thermal properties of the polymer were evaluated by using differential scanning calorimetry (DSC) and thermogravimetry (TG). In the DSC measurement

under nitrogen, glass transition temperature (Tg) was observed at 187 °C, which is lower than that of the commercially available PPO.[6] This is probably due to the lower molecular weight of the present polymer. In the TG measurement under nitrogen, the weight loss was hardly observed below 400 °C (the temperature at 5 weight % loss = 397 °C). These data indicate the polymer obtained by using **2** as a catalyst showed excellent thermal stability.

Oxidative Polymerization of Other 2,6-Disubstituted Phenols Catalyzed by Fe(III)-Salen Complex. Besides **1a**, other 2,6-disubstituted phenols, 2-allyl-6-methylphenol (**1b**), 2,6-diisopropylphenol (**1c**), 2,6-dimethoxyphenol (**1d**), and 2,6-diphenylphenol (**1e**) were used as monomer. In the polymerization of **1b** in 1,4-dioxane under the similar reaction conditions of **1a** (entry 4 in Table 1), the polymer with molecular weight of 8700 was obtained in 88 % yield. In the ^{1}H NMR spectrum of the polymer, five main peaks were observed: a singlet peak at δ 2.1 due to the methyl protons, peaks at δ 3.2, 5.0, and 5.8 ascribed to the protons of allyl group, and a singlet peak at δ 6.5 due to the aromatic protons. It is to be noted that the dimeric byproduct (DPQ derivative) was not detected in the methanol-insoluble part from **1b**. FT-IR spectrum showed peaks at 1205 and 1028 cm^{-1} due to the C-O-C vibration. These data indicate that the polymer from **1b** was of 1,4-oxyphenylene unit structure. From **1e**, an oligomer with molecular weight of 1500 was obtained in 59 % yield. ^{1}H NMR analysis of the product showed that the characteristic peak of the DPQ derivative from **1e** at δ 8.1 was much smaller than that due to the aromatic protons of the oxyphenylene unit, indicating that the catalysis of **2** afforded the PPO derivative from **1e**.

In the reaction of **1c**, the yield of the methanol-insoluble part was low (17 %). In the ^{1}H NMR spectrum of the product, observed was a characteristic singlet peak at δ 7.6 ascribed to the aromatic protons of the DPQ derivative from **1c**. From integrated ratio between this peak and peaks of isopropyl group, the DPQ content in the product

mixture was 80 %. IR spectrum showed a characteristic sharp peak at 1663 cm^{-1} due to the carbonyl moiety of DPQ. These data indicate that the main product by the oxidative coupling of **1c** by the Fe-salen complex was the DPQ derivative. This may be since the bulky substituent of **1c** prevented the polymer formation. A similar behavior was observed in using copper/amine catalyst.[5] From **1d**, an insoluble product was formed in 69 % yield. In the IR spectrum of the product, there was a characteristic strong peak at 1628 cm^{-1} ascribed to the carbonyl vibration, assuming the formation of the DPQ derivatives from the oxidative coupling of **1d** catalyzed by **2**.

Recently, another approach was developed to synthesize PPO derivatives by the polymerization of 3,5-dimethoxy-4-hydroxybenzoic acid (syringic acid) and 3,5-dimethyl-4-hydroxybenzoic acid by using peroxidase or laccase as a catalyst,[30] which is a new type of oxidative polymerization involving elimination of not only hydrogen but also carbon dioxide from the monomer. However, **2** did not induce the oxidative polymerization of syringic acid under the similar reaction conditions.

Synthesis of a soluble phenolic polymer by oxidative polymerization of bisphenol A using Fe(III)-salen complex as a catalyst. The enzymatically synthesized phenolic polymers often showed low solubility toward organic solvents, which restricts their applications as polymeric materials. This section deals with the oxidative polymerization of bisphenol A using **2** as a catalyst to produce the soluble phenolic polymer.

The oxidative polymerization of bisphenol A was performed by using Fe(III)-salen (**2**) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, at room temperature under air. As a polymerization solvent, 1,4-dioxane was used since it shows high solubility toward poly(bisphenol A) and is miscible with aqueous hydrogen peroxide solution. By the addition of hydrogen peroxide, the reaction solution turned dark-red and the reaction mixture was homogeneous during the

polymerization. This is in contrast with the previous study on the peroxidase-catalyzed polymerization of bisphenol A; the polymer precipitates were formed in the initial stage of the polymerization since the product polymer was insoluble in the reaction medium (a mixed solvent of buffer and water-miscible organic solvents such as methanol, acetone, and 1,4-dioxane).[31] After 3 h, the products were isolated by pouring the reaction mixture into a large amount of aqueous methanol (water/methanol = 50/50 (vol %)) to give the powdery polymer (yield 45 %). The polymer was soluble in polar organic solvents, i.e., chloroform, THF, methanol, acetone, and DMF. The polymer molecular weight and its index estimated by SEC analysis using THF eluent were 2700 and 2.4, respectively.

It is mentioned above that the oxidative polymerization of 2,6-dimethylphenol catalyzed by **2** afforded poly(phenylene oxide) along with a byproduct dimer of 3,5,3',5'-tetramethyl-4,4'-diphenoquinone, and the addition of pyridine efficiently suppressed the formation of the dimer and greatly improved the polymer yields. Thus, the polymerization of bisphenol A by **2** was performed in the presence of small amount of pyridine (0.020 mL per 1.0 mmol of the monomer) to give the polymer with molecular weight of 3400 in 69 %. These data indicate that the yield and molecular weight increased by the addition of pyridine. The role of the pyridine may be explained as follows. Bisphenol A is neutralized in situ by pyridine to produce a little amount of phenoxy anion having higher reactivity for oxidative couplings due to the larger complexation constant with metal catalyst.

Polymerization results are summarized in Table 5. The polymerization also proceeded in dimethoxyethane and THF. In all solvents, the yield and molecular weight values of the polymer obtained in the presence of pyridine were larger than those without pyridine. In using 1,4-dioxane, both values were the largest, indicating that 1,4-dioxane was the most suitable for the oxidative polymerization of bisphenol A by **2**. In this study, a very small amount of the catalyst (0.10 mol % for the monomer) afforded the polymer in moderate yields, showing the efficient catalysis of **2** for the

Table 5. Oxidative Polymerization of Bisphenol A catalyzed by **2** [a)]

Entry	Solvent	Pyridine (mL)	Yield [b)] (%)	Mn x 10^{-3} [c)]	Mw/Mn [c)]
1	Dimethoxyethane	0	18	2.4	2.0
2	Dimethoxyethane	0.1	42	2.8	2.1
3	1,4-Dioxane	0	45	2.7	2.4
4	1,4-Dioxane	0.1	69	3.4	2.9
5	THF	0	36	1.8	1.7
6	THF	0.1	45	2.2	1.8

[a)] Polymerization of bisphenol A (5.0 mmol) using **2** (5.0 μmol) and hydrogen peroxide as a catalyst and an oxidizing agent, respectively, in organic solvent (10 mL) at room temperature for 3 h under air. [b)] Methanol/water (50:50 vol%)-insoluble part. [c)] Determined by SEC.

bisphenol A polymerization.

Polymer structure was confirmed by ^{1}H NMR and IR spectroscopies. In the ^{1}H NMR spectrum, there were four broad peaks: δ 1.5 due to the methyl protons, δ 6.5 ascribed to the aromatic protons of ortho position, δ 6.9 due to the meta aromatic protons, and δ 9.1 due to the phenolic protons. In IR spectrum of the polymer (entry 4), observed were a broad peak centered at 3400 cm^{-1} due to the vibration of O-H linkage of phenolic group, peaks at 1218 and 1177 cm^{-1} ascribed to the asymmetric vibrations of the C-O-C linkage and to the C-OH vibration, and a peak at 1115 cm^{-1} due to the symmetric vibration of the ether. The spectrum pattern of the resulting polymer was similar to that obtained by using soybean peroxidase catalyst. These data show that the present polymer is composed of a mixture of phenylene and oxyphenylene units.

Fe-salen catalyst induced the polymerization of *p-tert*-butylphenol in 1,4-dioxane in the presence of pyridine to give the soluble polymer with molecular weight of 2200 in 83 % yield. Phenol and *m*-cresol were also polymerized under the similar reaction conditions to give the polymers showing low solubility toward organic solvents.

Thus, Fe-salen complex was found to be an efficient catalyst for the synthesis of soluble poly(bisphenol A) consisting of a mixture of phenylene and oxyphenylene units. The addition of pyridine increased the polymer yield and molecular weight. The application of the complex is not limited only to 2,6-disubstituted phenols.

Conclusion

Fe(III)-salen complex (**2**) was used as a new catalyst for oxidative polymerization of phenol derivatives. The author regards **2** as a model complex of peroxidase having a heme in the catalytic active site. The polymerization of 2,6-dimethylphenol catalyzed by **2** in the presence of an amine proceeded under mild reaction conditions to give the polymer exclusively consisting of 1,4-oxyphenylene unit. Complex **2** showed a very high catalytic activity for the oxidative polymerization. The molecular weight could be controlled by the solvent composition. 2-Allyl-6-methylphenol and 2,6-diphenylphenol were also polymerized by **2** to give PPO derivatives. Complex **2** catalyzed the polymerization of other phenols such as bisphenol A and *p-tert*-butylphenol to give soluble polymers.

References

1) Genies, E. M.; Boyle, A.; Lapkowski, M.; Tsintavis, C. *Synth. Met.* **1990**, *36*, 139.

2) Macdiarmid, A. G.; Epstein, A. J. *Faraday Discuss. Chem. Soc.* **1989**, *88*, 315.

3) Jasne, S. In *Encyclopedia of Polymer Science and Engineering*; 2nd ed., John Wiley & Sons: New York, 1986; Vol. 13, p 42.

4) Hay, A. S. *J. Polym. Sci.*, **1962**, *58*, 581.

5) Hay, A. S. *J. Polym. Sci., Polym. Chem. Ed.* **1998**, *36*, 505.

6) Aycock, D.; Abolins, V.; White, D. M. In *Encyclopedia of Polymer Science and Engineering*; 2nd ed.; John Wiley & Sons: New York, 1986; Vol. 11, p 45.

7) Zhang, W.; Loebach, J. L.; Wilson, S. R.; Jacobson, E. N. *J. Am. Chem. Soc.* **1990**, *112*, 2801.

8) Finney, N. S.; Pospisil, P. J.; Chang, S.; Palucki, M.; Konsler, R. G.; Hansen, K. B.; Jacobson, E. N. *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1720.

9) Sasaki, H.; Irie, R.; Katsuki, T. *Synlett.* **1993**, 300.

10) J. Dao, D. Benoit, and C. J. Hawker, *J. Polym. Sci., Polym. Chem. Ed.*, *36*, 2161 (1998).

11) De, B. B.; Lohray, B. B.; Sivaram, S.; Dhal, P. K. *Tetrahedron Asymm.* **1995**, *6*, 2105.

12) De, B. B.; Lohray, B. B.; S. Sivaram; Dhal, P. K. *J. Polym. Sci., Polym. Chem. Ed.* **1997**, *35*, 1809.

13) Corrazza, F.; Floriani, C.; Zehnder, M. *J. Chem. Sci., Dalton Trans.* **1987**, 709.

14) Mukherjee, R. N.; Stack, T. D. P.; Holm, R. H. *J. Am. Chem. Soc.* **1988**, *110*, 1850.

15) Davies, J. E.; Gatehouse, B. M. *Acta Crystallogr.* **1973**, *B29*, 1934.

16) Strauss, S. M.; Pawlik, M. J.; Skowyra, J.; Kennedy, J. R.; Anderson, O. P.;

Spartalian, K.; Dye, J. L. *Inorg. Chem.* **1987**, *26*, 724.

17) Swepston, P. N.; Ibers, J. A.; *Acta Crystallogr.* **1985**, *C41*, 671.

18) Dick, D. L.; Rao, T. V. S.; Sukumaran, D.; Lawrence, D. S. *J. Am. Chem. Soc.* **1992**, *114*, 2664.

19) Rivera, M.; Caignan, G. A.; Astashkin, A. V.; Raitsimring, A. M.; Shokhireva, T. Kh.; Walker, F. A. *J. Am. Chem. Soc.* **2002**, *124*, 6077.

20) Sanders-Loehr, J. In *Iron Carriers and Iron Proteins*; Loher, T. M., Ed.; VCH: New York, 1989; p 373.

21) Que, L., Jr.; Scarrow, R. C. In *Metal Clusters in Proteins*; Que, L., Jr., Ed.; American Chemical Society: Washington, DC, 1988; p 302.

22) Kurtz, D. M., Jr. *Chem. Rev.* **1990**, *90*, 585.

23) Tsuchida, E.; Nishide, H.; Nishiyama, T. *Makromol. Chem.* **1975**, *176*, 1349.

24) Pfeiffer, P.; Breith, E.; Lübbe, E.; Tumaki, T. *Ann.* **1933**, *503*, 84.

25) van Aert, H. A. M.; van Genderen, M. H. P.; van Steenpaal, G. J. M. L.; Nelissen, L.; Meijer, E. W.; Liska, J. *Macromolecules* **1997**, *30*, 6056.

26) Finkbeiner, H.; Hay, A. S.; Blanchard, H. S.; Endres, G. F. *J. Org. Chem.* **1966**, *31*, 549.

27) Price, C. C. *ACS Symp. Ser.* **1975**, *6*, 1.

28) van Aert, H. A. M.; Venderbosch, R. W.; van Genderen, M. H. P.; Lemstra, P. J.; Meijer, E. W. *J. Macromol. Sci.-Pure Appl. Chem.*, **1995**, *A32*, 515.

29) Ikeda, R.; Sugihara, J.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 8702.

30) Ikeda, R.; Uyama, H.; Kobayashi, S. *Macromolecules* **1996**, *29*, 3053.

31) Kobayashi, S; Uyama, H; Ushiwata, T; Uchiyama, T; Sugihara, J; Kurioka, H. Macromol. Chem. Phys. **1998**, *199*, 777.

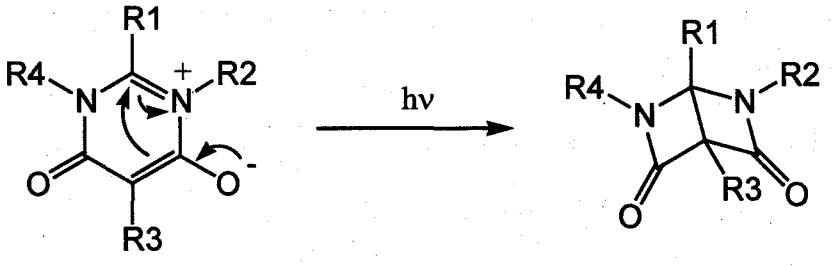
Chapter 6

Synthesis of a Phenolic Polymer with a Mesoionic 6-Oxo-1,6-dihydropyrimidin-3-ium-4-olate as Pendant Group and Its Photochemical Behavior

Introduction

Mesoionic 6-oxo-1,6-dihydropyrimidin-3-ium-4-olates (**1**) were first synthesized in 1971[1,2] and the interest in that 6-membered mesoionic heterocycles was originally focused on 1,4-dipolar cycloadditions with olefins and alkynes.[3-6] It was also shown that these compounds are photosensitive and undergo an intramolecular cycloaddition which leads to the formation of bis(β-lactams) (**2**)[7] as pictured in Scheme 1.

Scheme 1.



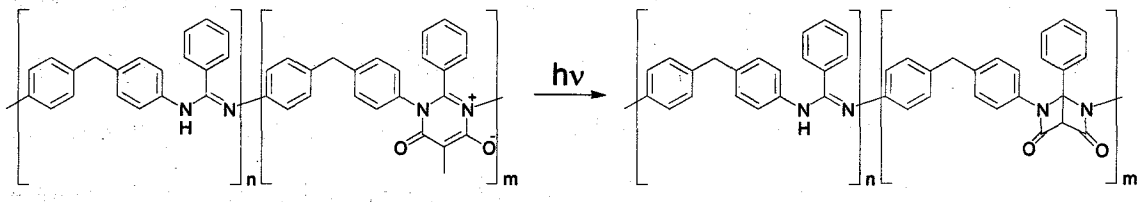
1 **2**

With the change of the chemical structure, one can expect also changes of the physical properties such as color, specific volume, dipole moment, and refractive index. For this reason, it is interesting to prepare polymers containing 6-oxo-1,6-dihydropyrimidin-3-ium-4-olates, which can be used as photosensitive

materials.

Up to now, different types of mesoions containing styrenic[8-10] or methacryl[11] moiety have been synthesized and polymerized by Ritter et al. Recently, they also prepared polymers containing mesoionic groups in the main chain.[12,13] In addition, the changes in film thickness and refractive index on irradiation of a spin coated polymer film with a mesoionic group in the main chain (Scheme 2) was analyzed by waveguide spectroscopy.[13] This method is based on the selective excitation of waveguide modes by prisms or diffraction gratings. [14-16]

Scheme 2.



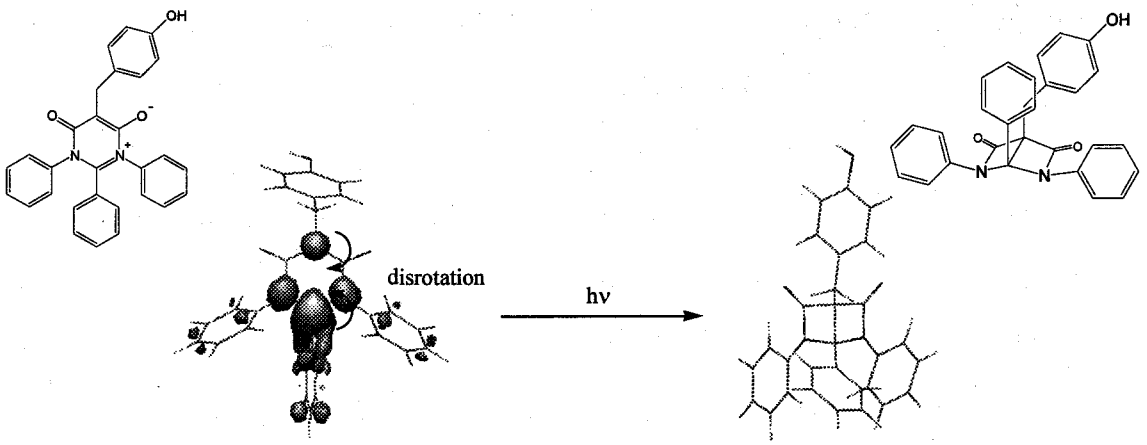
It was shown that laser irradiation caused anisotropic changes in the refractive index and reduction of the film thickness,[16] which is controlled by a complicated interplay of the anisotropy at the beginning caused by the spin coating process, and the cyclization and reorientation kinetics in both polarization directions of the polymeric structure. For that reason, polymers with mesoionic groups are interesting materials for optical data storage or other optical devices.

Recently, it was found that a binuclear iron salen complex, which has five-coordinate iron (III) at the center, efficiently catalyzed oxidative polymerization of a wide variety of phenols to give phenolic polymers.[17-21] Especially, the polymerization of *p*-substituted phenols gave soluble polymers, whose properties are required for some applications. Resultant polymers often consisted of a mixture of phenylene and oxyphenylene units and the former unit may provide a unique photochemical behavior when UV light was irradiated unlike polymers containing no absorbance in the range of

the laser which was used for irradiation.

In this chapter, a phenolic polymer bearing the photosensitive mesoionic group as pendant group was synthesized and its photoreactivity was investigated. Expected photocyclization of the mesoion is depicted in scheme 3.

Scheme 3.



LUMO

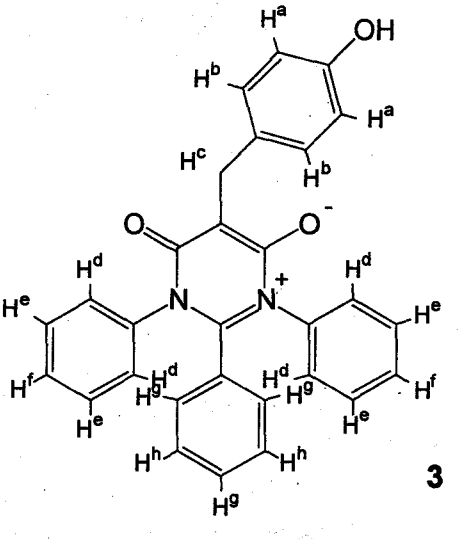
Experimental section

Materials. *N,N*-Diphenylformamidine[22] and binuclear iron salen complex[23] were synthesized according to the literatures. All other reagents were commercially available and used without further purification.

4-Benzyloxybenzyl malonic acid (**7**): Diethyl malonate (12 g, 75 mmol) and sodium ethoxide (5.1 g, 75 mmol) were solubilized in 40 ml of ethanol at room temperature under gentle stirring. The homogeneous solution was cooled to 0 °C and 4-benzyloxybenzyl chloride (**5**) (5.8 g, 25 mmol) was added gradually. The mixture was stirred at room temperature for 8 h, and then acidified by dilute HCl solution, followed

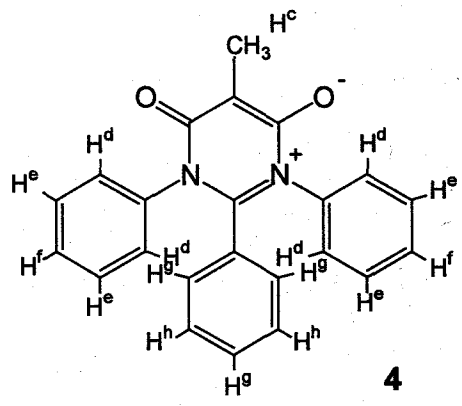
by extraction with diethyl ether. After evaporation of diethyl ether and distillation of most part of unreacted diethyl malonate under vacuum at 170 °C, the residue was subjected to flash chromatography using hexane/ethyl acetate as eluent. The obtained sticky product was dissolved in aqueous ethanol/NaOH solution and washed by diethyl ether. The aqueous phase was acidified by dilute HCl solution, followed by extraction with diethyl ether. Evaporation of the organic phase and drying in vacuum yielded 5.0 g of white precipitate (**7**) (yield 60 %): ^{1}H NMR (DMSO-d_6): δ 2.95 (d, 2H, CH_2), 3.51 (t, 1H, CH), 5.05 (s, 2H, OCH_2), 6.90-7.45 (m, 9H, Ar).

Mesoionic 5-(4-hydroxybenzyl)-6-oxo-1,2,3-triphenyl-1,6-dihydropyrimidin-3-ium-4-olate (**3**): To a solution of *N,N*-diphenylbenzamidine (**8**) (1.85 g, 6.80 mmol) and dicyclohexylcarbodiimide (DCC) (2.81 g, 13.6 mmol) in 8 mL of dry *N,N*-dimethylformamide (DMF), **7** (2.02 g, 6.73 mmol) was gradually added at 0 °C under stirring. After 1 h, the color of the reaction mixture turned to bright yellow. During the reaction at room temperature for 24 h, urea was precipitated, which was removed by filtration. The filtrate was concentrated by evaporation under reduced pressure. Compound **9** was crystallized by adding chloroform (yield 62 %) and converted to **8** by deprotection of benzyl group with $Pd(OH)_2$ catalyst in acetonitrile (yield 83 %): ^{1}H NMR (DMSO-d_6): δ 3.56 (s, 2H, H^c), 6.61 (d, 2H, H^a), 6.96 (m, 3H, H^g), 7.15 (t, 1H, H^f), 7.18 (d, 2H, H^b), 7.22 (t, 2H, H^e), 7.28 (d, 2H, H^d) 7.42 (m, 2H, H^h); MS (FAB, glycerol matrix): m/z 447 [$(M+H)^+$].



3

Mesoionic 5-methyl-6-oxo-1,2,3-triphenyl-1,6-dihydropyrimidin-3-ium-4-olate (**4**): To a solution of *N,N*-diphenylbenzamidine (**8**) (1.00 g, 3.67 mmol) and DCC (1.51 g, 7.34 mmol) in 7.5 mL of dry dichloromethane, methylmalonic acid (0.43 g, 3.67 mmol) was gradually added under stirring over a period of 10 min, whereby the reaction mixture turned to bright yellow. After 1 h, the precipitated urea was filtered off. The filtrate was concentrated by evaporation under reduced pressure and the desired mesoionic compound (**4**) was crystallized by adding diethyl ether and petroleum ether (yield 62 %): ^{1}H NMR (DMSO-d_6): δ 1.87 (s, 3H, H^c), 6.90-7.00 (m, 3H, H^g), 7.09-7.14 (m, 10H, $H^{d,e,f}$), 7.15-7.44 (m, 2H, H^h); IR (ATR): 3054 (Ar. C-H), 2918, 2859 (aliph. C-H), 1640 (C=O), 1595 (Ar. C=C), further signals at 1489, 1445, 1382, 1344, 1324, 1290, 1158, 1068, 1026, 1003, 971, 933, 833, 748, 713, 694; UV (DMF): λ_{max} [nm] (log ε) = 268 (3.90), 348 (3.29); MS (FD): m/z 354 (100) [M^+].



4

Polymerization of monomer 8. The iron salen complex (5.0 mg, 7.5 μmol) and **3** (450 mg, 1.0 mmol) were dissolved in pyridine (5.0 mL). Ten % hydrogen peroxide (0.51 mL, 1.5 mmol) was added dropwise to the mixture for 2 h at room temperature under air. After the subsequent stirring of the reaction mixture for an hour, the resulting solution was poured into a large amount of methanol/water (50:50 vol%). Obtained precipitates were collected by centrifugation and followed by washing with methanol/water (50:50 vol%) repeatedly. The precipitates were dried in vacuum to give 241 mg of the polymer (yield 54 %, Mn = 4000): IR (ATR): 3133, 3062 (Ar. C-H), 2977, 2932 (aliph. C-H), 1643 (C=O), 1596 (Ar. C=C), further signals at 1692, 1492, 1443, 1315, 1273, 1210, 1105, 1068, 1026, 1003, 834, 753, 712, 691; UV (DMF): λ_{max} [nm]

(log ε) = 268 (4.15).

Measurement. ^{1}H NMR spectra were recorded on a 400 MHz Bruker DPX-400 or 200 MHz Bruker AC-200 spectrometer. IR spectra were measured on a Nicolet 5SXB FT-IR spectrometer equipped with a MCT detector and a Specac golden gate diamond ATR unit. UV spectra were performed on a Unicam UV 540 spectrometer. FAB mass measurement was carried out using a JEOL high performance JMS-HX110 mass spectrometer. FD mass spectrometry was performed on a Finnigan MAT 95, emitter heat-rate: 10 mA / min. SEC analysis was carried out using a TOSOH SC8020 apparatus with a refractive index (RI) detector at 35°C under the following conditions: TSKgel α-M column and 0.1M LiCl/DMF eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. The polymeric films were prepared using a 15 wt.% solution of the polymer in a 1:1 mixture of DMF and 1-methyl-2-pyrrolidone on a BLE Delta 10 spincoater with exhaust air separation (600 rpm, highest acceleration, 5 min., followed by 2000 rpm, lowest acceleration, 10 min.). The films were dried in vacuum at 60°C for 48 h. Waveguide mode spectroscopy experiments were carried out using a home built spectrometer (Figure 1).

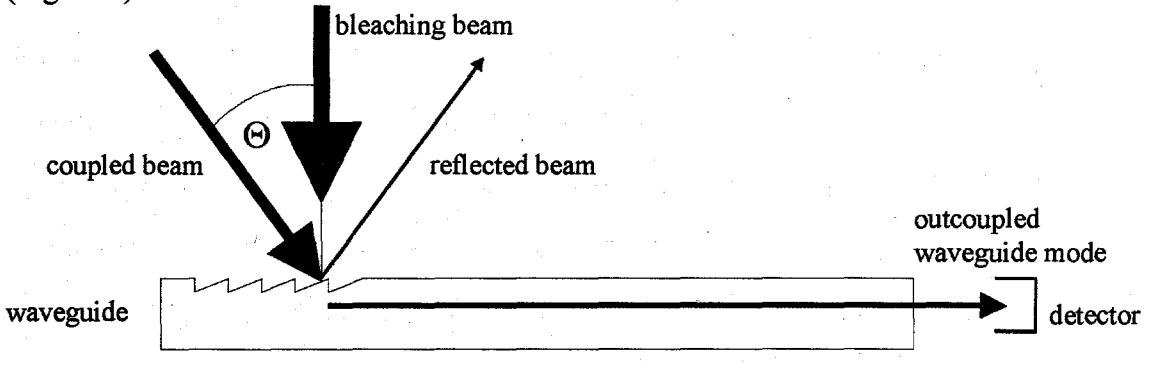


Figure 1. Setup for waveguide mode spectroscopy with simultaneous irradiation of the mesoionic polymer sample.

A grating with a periodicity Λ of 597 nm ion etched into the substrate[24] was used to couple a HeNe laser beam (λ = 632.8 nm) in s- (TE) and p-polarization (TM)

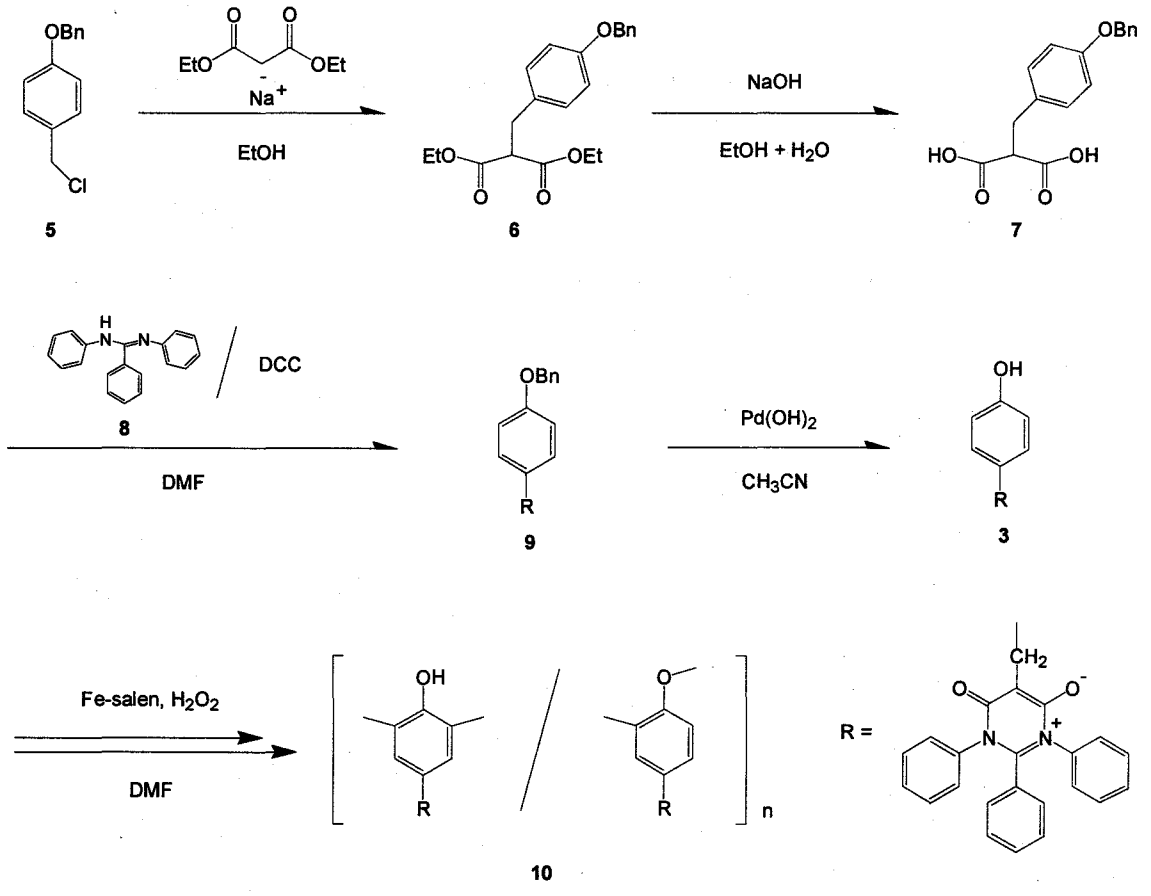
from the air into the waveguide. The waveguide mode spectrum was obtained by scanning the angle of incidence of the incoming laser beam onto the grating while measuring the out-coupled power with a photo detector situated at the end of the waveguide.[14] From the coupling angles and the known parameter of the grating the effective refractive indices of all modes N_{ij} was calculated according to equation (1).

$$N_{i,j} = n_c \cdot \sin \alpha + m \cdot \lambda/\Lambda \quad (1)$$

n_c is the refractive index of the cladding (here air with $n_c = 1$), λ the wavelength of the coupled light, Λ the grating period, and m the diffraction order. With the knowledge of the optical constants of the substrate, the thickness and refractive index for both polarization directions were calculated iteratively as long as at least two modes for each direction could be detected. The irradiation of the film was carried out with the focused light of a mercury vapor short arc lamp (Type Osram HBO 100W) at a power of 50 W, filtered by a water filled IR filter and a 2 mm Schott UG-1 filter (365 nm max. transmission).

Results and discussion

Scheme 4.



Polymer synthesis. The oxidative polymerization of **3** was carried out using hydrogen peroxide as an oxidant in pyridine. The resulting polymer was soluble in DMF, dimethyl sulfoxide, and pyridine, but insoluble in acetone, methanol, hexane, and water. The UV spectrum of low molecular weight model compound (**4**) shows a strong and narrow absorption band at 268 nm and a broader absorption at 348 nm, coming from the mesoionic HOMO-LUMO transition. In the polymeric product, these two bands are overlayed by a further signal with a nearly exponentional decay to longer wavelengths. This absorbance is typical for polymeric phenols and is probably due to extended conjugated system based on the phenylene linkage.

Photochemical behavior of the polymer. In order to proof the applicability of the mesoionic phenolic polymer as a photosensitive material, films with a thickness at about 1400 nm were prepared with a spincoater. Using a substrate with a grating, the compound can be used as a waveguide, whereby the film thickness and refractive index can be calculated for both polarization directions. Before irradiation, the refractive index was determined to be 1.620 in TE direction, which is parallel to the surface of the substrate and 1.617 in TM direction, being perpendicular to the surface. This values differ only very little and are within the error limit of the measurement, so the film seems to be almost isotropic. This result differs from that obtained from the polymer, bearing the mesoionic groups in the main chain,[16] which can be explained by an increased mobility of the mesoionic groups. The thickness of the film was calculated to be 1370-1380 nm, also identical within the error limit. The sample was irradiated for about 14 h with the filtered light of a mercury vapor lamp resulting a maximum at 365 nm, which is proper to the HOMO-LUMO transition. At different times, the waveguide mode spectrum was scanned and the refractive index and film thickness were calculated (Figure 2 and 3).

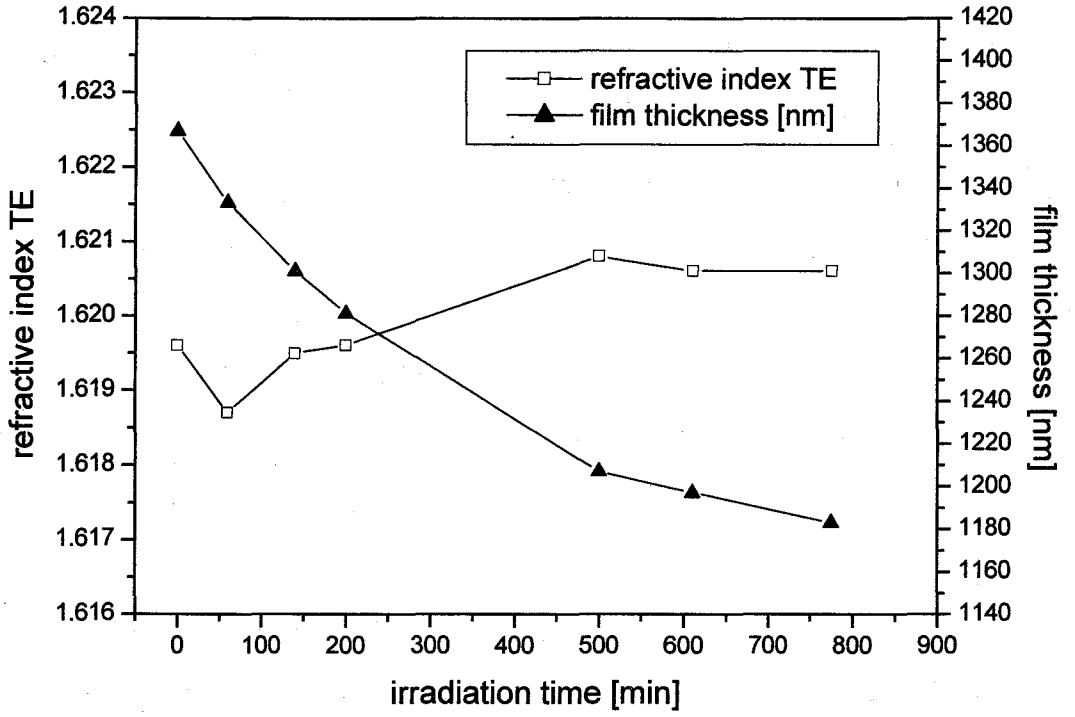


Figure 2. Time-development of the refractive index and the film thickness measured with s-polarized light (TE). Errors : $\Delta n = \pm 0.004$ and $\Delta d = \pm 40$ nm.

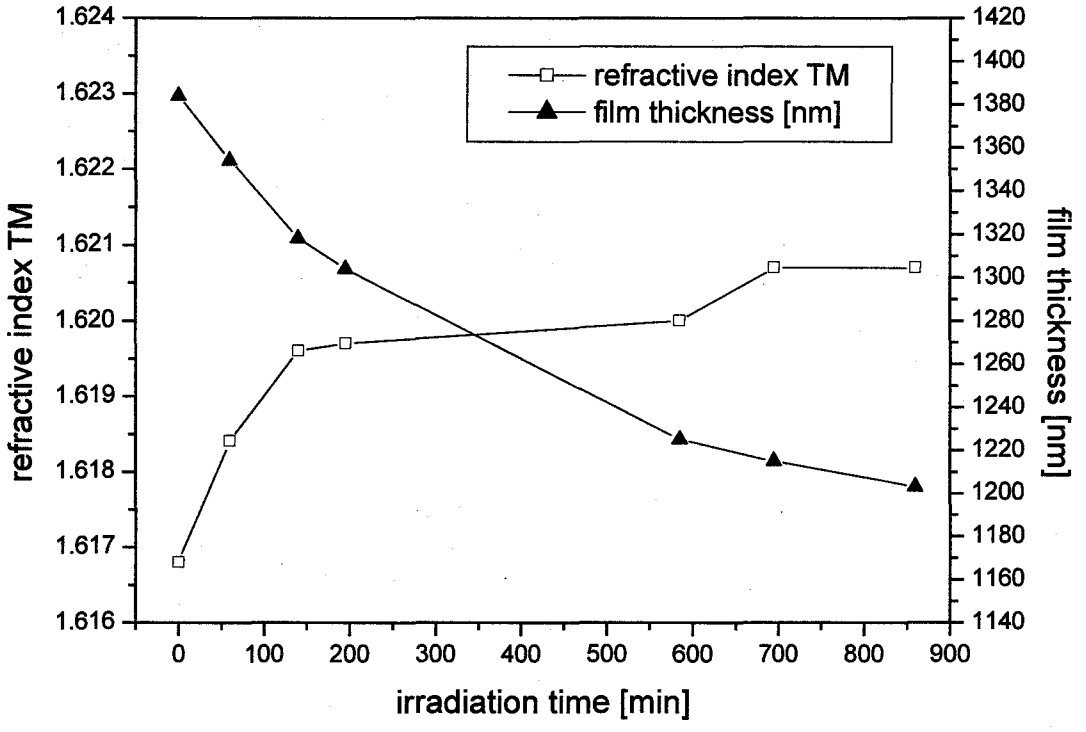


Figure 3. Time-development of the refractive index and the film thickness measured with p-polarized light (TM). Errors : $\Delta n = \pm 0.004$ and $\Delta d = \pm 40$ nm.

Whereas the refractive index remains nearly constant within the experimental errors and does not show any anisotropic behavior, a strong decrease of the film thickness could be observed. After irradiation, the film was still soluble in THF, so no crosslinking has taken place. An IR spectrum was recorded (Figure 5) and compared to that measured before irradiation (Figure 4). The spectra show distinct changes in the region of the C=O vibrations. In lieu of the mesoionic C=O vibration at 1643 cm^{-1}, a strong absorption at 1693 cm^{-1} appears, which can be assigned to the C=O vibration of the bis(β-lactam). The other parts of the IR spectra show only minor changes, which is a strong hint, that the shown effect is based on the photocyclization of the mesoionic groups and not on changes of the phenol polymer backbone.

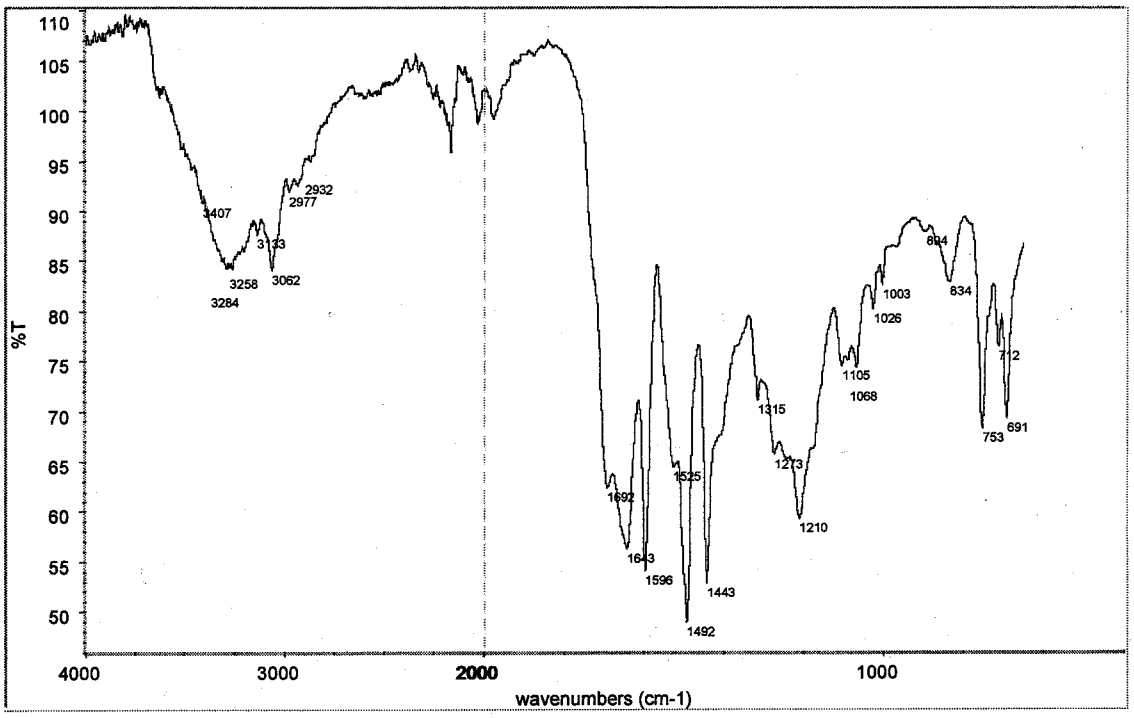


Figure 4. IR spectrum of **10** before irradiation.

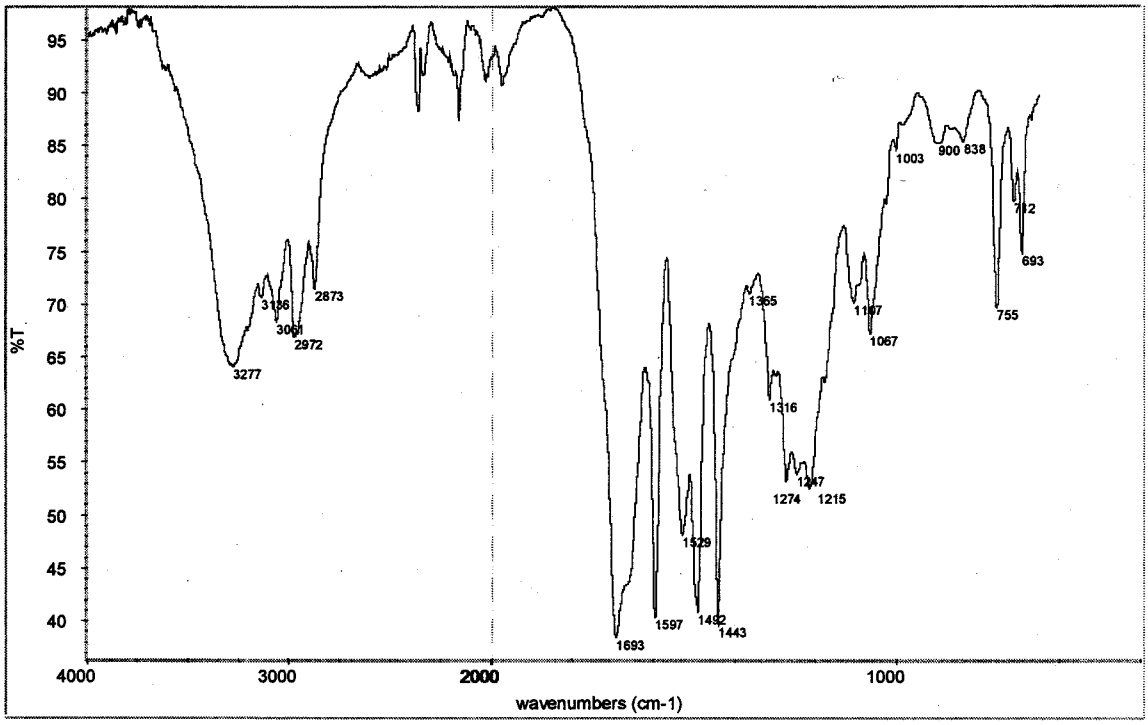


Figure 5. IR spectrum of **10** after irradiation.

The strong decrease in the film thickness can be explained by reorientation of the polymer conformations caused by the different steric demand and the reduced dipolaric character of the bis(β-lactam) groups compared to the mesoionic groups. With a decrease of the film thickness, the density of the material is increased. Due to theoretical considerations and experimental results of other samples,[13] a reduction of the molar polarizability can be assumed. In the described sample, the effects of reduction of the molar polarizability and the increase of the density equalize or cancel each other to some extent, so that the refractive index nearly remains constant.

Conclusion

A mesoionic phenolic polymer, which has 6-oxo-1,6-dihydropyrimidin-3-ium-4-olate moiety as pendant group, was synthesized by an oxidative polymerization using

the iron salen complex as a catalyst. The resultant polymer was soluble in polar organic solvents and had UV absorbance even in the range of visible light due to the structure of phenolic polymer. The polymer can be processed as a film, which was proofed to be photosensitive. Irradiation causes a change of the mesoionic structure to a bis(β-lactam) structure, which could be observed by IR spectra. In association with that change, a strong decrease of the film thickness without refractive index changes could be determined by waveguide spectroscopy.

References

1) Potts, K. T.; Sorm, M. *J. Org. Chem.* **1971**, *36*, 8.

2) Kappe, T.; Lube, W. *Monatsh. Chemie* **1971**, *102*, 781.

3) Friedrichsen, W.; Kappe, T.; Böttcher, A. *Heterocycles* **1982**, *19*, 1083.

4) Gotthardt, H.; Schenk, K. H. *Chem. Ber.* **1985**, *118*, 2079.

5) Gotthardt, H.; Blum, J. *Chem. Ber.* **1987**, *120*, 115.

6) Gotthardt, H.; Blum, J. *Chem. Ber.* **1986**, *119*, 3247.

7) Gotthardt, H.; Schenk, K. H. *J. Chem. Soc., Chem. Commun.* **1986**, 687.

8) Ritter, H.; Sperber, R.; Weißhuhn, C. M. *Macromol. Chem. Phys.* **1994**, *195*, 3823.

9) Ritter, H.; Sperber, R. *Macromol. Rapid Commun.* **1995**, *16*, 407.

10) Deutschmann, T.; Ritter, H. *Macromol. Chem. Phys.* **2000**, *201*, 1200.

11) Theis, A.; Ritter, H. *Des. Monomers Polym.* **2001**, *4*, 177.

12) Deutschmann, T.; Ritter, H. *Macromol. Rapid Commun.* **1996**, *17*, 723.

13) Theis, A.; Ritter, H.; Böhme, F.; Klinger, C.; Menges, B.; Mittler, S. *Chem. Mat.* **2002**, *14*, 2109.

14) Lee, T.-M.; Mittler-Neher, S.; Neher, D.; Stegeman, G. I.; Roux, C.; Leclerc,

M. *Optical Materials* **1992**, *1*, 65.

15) Ihlein, G.; Menges, B.; Mittler-Neher, S. *Optical Materials* **1995**, *4*, 685.

16) Paul, S.; Halle, O.; Menges, B.; Einsiedel, H.; Müllen, K.; Knoll, W.; Mittler-Neher, S. *Thin Solid Films* **1996**, *288*, 150.

17) Tonami, H.; Uyama, H.; Kobayashi, S.; Higashimura, H.; Oguchi, T. *J. Macromol. Sci.-Pure Appl. Chem.* **1999**, *A36*, 719.

18) Tonami, H.; Uyama, H.; Higashimura, H.; Oguchi, T.; Kobayashi, S. *Polym. Bull.* **1999**, *42*, 125.

19) Ikeda, R.; Tanaka, H.; Uyama, H.; Kobayashi, S. *Macromolecules* **2000**, *33*, 6648.

20) Ikeda, R.; Tanaka, H.; Uyama, H.; Kobayashi, S. *Macromol. Rapid Commun.* **2000**, *21*, 496.

21) Ikeda, R.; Tanaka, H.; Uyama, H.; Kobayashi, S. *Polymer* **2002**, *43*, 3475.

22) Taylor, E. C.; Ehrhart, W. A. *J. Org. Chem.* **1963**, *28*, 1108.

23) Pfeiffer, P.; Breith, E.; Lübbe, E.; Tumaki, T. *Ann.* **1933**, *503*, 84.

24) Mai, X.; Moshrefzadeh, R.; Gibson, U. J.; Stegeman, G. I.; Seaton, C. T. *Applied Optics* **1985**, *24*, 3155.

Chapter 7

Oxidative Grafting of Phenolic Polymers onto Phenol-Containing Cellulose: Synthesis of Cellulose-Phenolic Polymer Hybrid

Introduction

Oxidative polymerization of phenols has been intensively studied to develop economically and ecologically superior process of polymer synthesis.[1-14] Most of them deal with homopolymerization regardless of the variety of reaction system. For example, several kinds of enzyme catalysts were used to produce alternatives of conventional phenolic resins in various media.

Modification of cellulose has been eagerly investigated for scientific and practical interest especially because cellulose is the most abundant polymer available and a renewable resource by nature. From the commercial and environmental viewpoints, covalent modification of cellulose is expected to be one of the most promising routes to afford a new class of high-performance green polymers. Grafting of polymers onto cellulose has been studied as one way to improve the properties and/or provide novel functions,[15-22] in which many of the side chain were vinyl polymers and aliphatic polyesters.

Main components of wood are cellulose, hemicellulose, and lignin. In the present chapter, the author has synthesized hybrid polymers consisting of two components of them, cellulose and a lignin-model polymer, an enzymatically synthesized phenolic polymer, which may be regarded as an artificial wood polymer. The hybrid polymers were synthesized by an iron salen-catalyzed oxidative grafting of the phenolic polymers onto a phenol-containing cellulose derivative. To my best

knowledge, this study is the first example of grafting by an oxidative cross-coupling between two polymers.

Experimental Section

Materials. Phenolic polymers,[4,13] tosyl cellulose[23] and the iron salen complex[24] were synthesized according to the literatures. The other reagents and solvents were commercially available and used as received.

Preparation of 2. Tosyl cellulose (3.5 g, DS = 0.92) was swelled in 350 ml of *N,N*-dimethylformamide (DMF) at room temperature. After adding 3.5 g of sodium 4-hydroxyphenyl acetate and purge, the solution was kept at 100 °C under argon to produce a clear solution. After 2 h, DMF was removed by evaporation under reduced pressure, and then the residue was washed with water and subsequently methanol repeatedly, followed by drying in vacuo (2.5 g). ^{1}H NMR (DMSO-d_6): δ 3.0-5.6 (br, cellulose backbone and CH_2Ar), 6.5-6.8 (br, Ar), 6.9-7.2 (br, Ar), 9.1-9.4 (br, ArOH); Anal. Found: C, 51.89; H, 5.36; S, 1.76. Syntheses of other phenol-containing celluloses **3** and **4** are similar to that of **2**. ^{1}H NMR of **3** (DMSO-d_6): δ 2.4-2.6 (br, CH_2Ar), 2.6-2.8 (br, CH_2CO), 3.0-5.6 (br, cellulose backbone), 6.5-6.8 (br, Ar), 6.9-7.2 (br, Ar), 9.1-9.4 (br, ArOH); ^{1}H NMR of **4** (DMSO-d_6): δ 3.0-5.6 (br, cellulose backbone), 6.6-6.9 (br, Ar), 7.6-7.9 (br, Ar), 10.1-10.4 (br, ArOH).

Grafting of poly(bisphenol A). The following is a typical procedure (entry 14 in Table 1). The phenol-containing cellulose **2** (17 mg), the iron salen complex (0.66 mg) and poly(bisphenol A) (34.2 mg) were dissolved in pyridine (2 mL) at room temperature under air. Hydrogen peroxide (12 μmol) was added with stirring. Every 20

min, 3.8 mg of poly(bisphenol A) and 12 mmol of hydrogen peroxide were added to the reaction mixture for three times. After 23 h of further stirring, the reaction mixture was poured into an excess amount of methanol to remove homo-coupled poly(bisphenol A) and the catalyst. The precipitate was collected by centrifugation and washed with methanol, followed by drying in vacuo (39 mg). ^{1}H NMR (DMSO-d_6): δ 1.0-1.7 (br, CH_3) 3.0-5.6 (br, cellulose backbone), 6.3-7.2 (br, Ar), 9.0-9.4 (br, ArOH); Anal. Found: C, 66.79; H, 5.73; S, 0.64.

Hydrolysis of the hybrids. The isolated hybrid (15 mg) was dissolved in DMF (4.0 mL). Sodium methoxide in methanol (28 %, 50 μL) was added at room temperature under nitrogen atmosphere. After 24 h of stirring, the solution was poured into an excess amount of water and acidified by dilute HCl solution. The precipitate was collected by centrifugation and washed with water, followed by drying in vacuo (6 mg, entry 12 in Table 1).

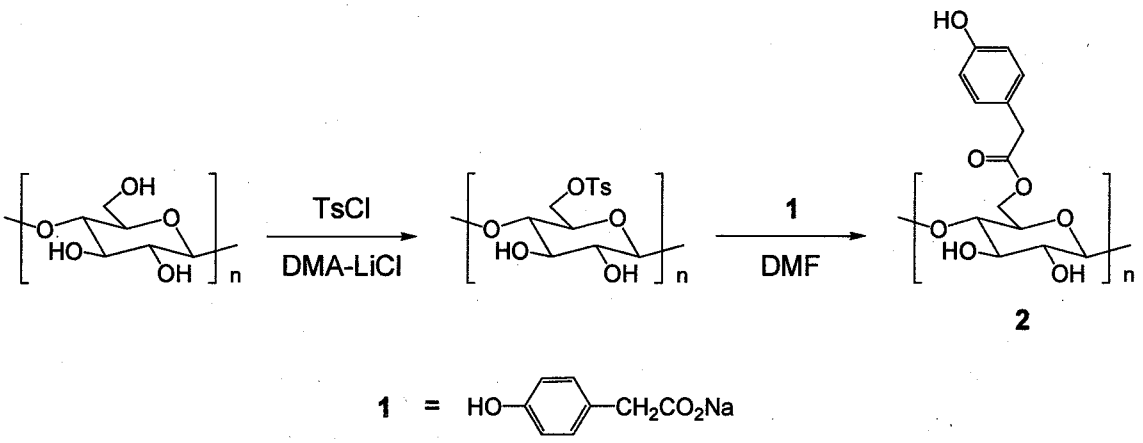
Measurements. ^{1}H NMR spectra were recorded on a 400 MHz Bruker DPX-400 instrument. SEC analyses were carried out using a TOSOH SC8020 apparatus with a UV detector at 35°C under the following conditions: TSKgel α-M column and 0.1M LiCl/DMF eluent at a flow rate of 1.0 mL/min. The calibration curves for SEC analysis were obtained using polystyrene standards. UV spectra were measured on a Hitachi U-2001 spectrometer.

Results and Discussion

Oxidative grafting of poly(bisphenol A) onto a phenol-containing cellulose. Cellulose-phenolic polymer hybrid was synthesized via an oxidative coupling of phenol

moiety between cellulose and the phenolic polymer. Cellulose has no phenolic group; thus phenol-containing cellulose derivative was designed and prepared as shown in Scheme 1. At first, tosylation of cellulose was performed in *N,N*-dimethylacetamide (DMA) / LiCl,[23] and subsequently a phenol group was introduced in cellulose by reaction of tosyl cellulose with sodium 4-hydroxyphenyl acetate (**1**) to produce a phenol-containing cellulose (**2**). The degree of substitution (DS) of the phenol group was determined by elemental analysis and ^{1}H NMR as 0.75, considering sulfur amount in **1** and aromatic protons of phenol group, respectively. The phenol-containing cellulose **2** was soluble in highly polar organic solvents as DMF, dimethyl sulfoxide (DMSO) and pyridine.

Scheme 1.



In this chapter, poly(bisphenol A)[13] was mainly used as a phenolic polymer, which shows high solubility toward polar organic solvents for the oxidative coupling. An iron salen-catalyzed grafting of poly(bisphenol A) onto **2** was performed in pyridine at room temperature under air. The reaction proceeded by adding aqueous hydrogen peroxide dropwise. After each drop, a small portion of the reaction mixture was analyzed by size exclusion chromatography (SEC) with UV detector at 340 nm (Figure 1). This wavelength was chosen to detect only coupling products containing

poly(bisphenol A) chains because **2** has no absorption at 340 nm; one unimodal peak due to poly(bisphenol A) was observed at retention time of ca. 17 min before reaction (Figure 1(a)).

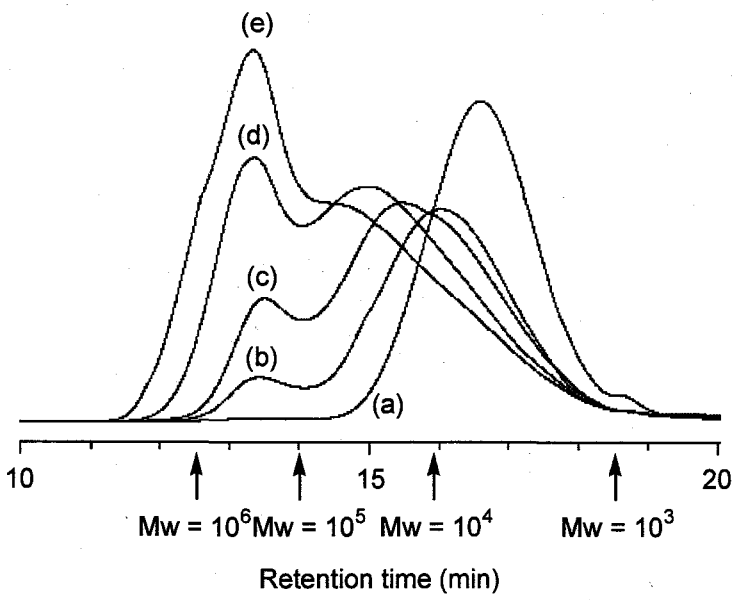


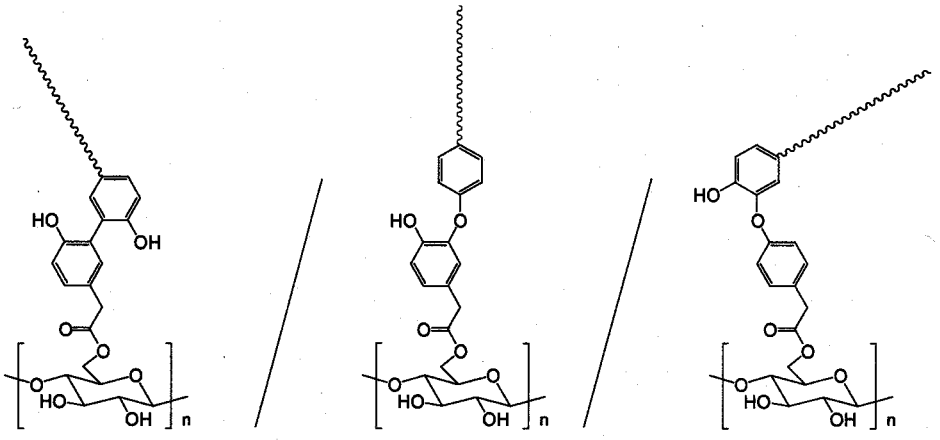
Figure 1. GPC traces of the reaction mixtures monitored by UV detector (340 nm). (a) Before reaction (poly(bisphenol A)), (b) H_2O_2 12 μmol (entry 11 in Table 1), (c) H_2O_2 24 μmol (entry 12 in Table 1), (d) H_2O_2 36 μmol (entry 13 in Table 1), (e) H_2O_2 48 μmol (entry 14 in Table 1).

In the present coupling, both the phenolic polymer and **2** have a reactive phenol group. Therefore, the cross-coupling between both polymers as well as homo-coupling of the phenolic polymer or **2** simultaneously take place. For the efficient production of the hybrid polymer, it is necessary to examine the reaction conditions for the selective cross-coupling. The present reaction proceeds via addition of hydrogen peroxide. When a small amount of hydrogen peroxide was added, the molecular weight of poly(bisphenol A) increased due to homo-coupling and at the same time small peak was observed at retention time of ca. 13 min (Figure 1(b)). This is due to the product formed by cross-coupling between poly(bisphenol A) and the phenol-containing cellulose,

which is hybrid. As the reaction proceeded by further addition of hydrogen peroxide, the amount of the hybrid increased gradually (Figure 1). This result indicates that poly(bisphenol A) was grafted onto **2**, accompanying homo-coupling of poly(bisphenol A). It is likely that homo-coupling of **2** was suppressed under this reaction condition, since only one drop addition of hydrogen peroxide to **2** caused gelation in the absence of poly(bisphenol A).

The hybrid was purely isolated by pouring the reaction mixture into an excess amount of methanol; after the reprecipitation, only the peak of the higher molecular weight product was detected in the SEC chart. The structure of the hybrid was confirmed by ^{1}H NMR, UV and elemental analysis. The proposed structures of the linkage between the phenol group formed by the oxidative grafting is depicted in Chart 1. When tosyl cellulose was used instead of **2**, the hybrid was not obtained. Furthermore, the control experiment showed that the iron salen complex and hydrogen peroxide were absolutely necessary for producing the hybrid. These data clearly indicate the phenolic group of the backbone is critical to produce the hybrid and non-covalent bond such as Van der Waals interaction and/or hydrogen bond between cellulose and phenolic polymer are not essential for the hybrid formation.

Chart 1.



The hybrid synthesis was summarized in Table 1. When a small amount of

poly(bisphenol A) was used (entry 6), gelation took place as soon as hydrogen peroxide was added, indicating homo-coupling of **2** was superior. A large amount of poly(bisphenol A) resulted only in homo-coupling of poly(bisphenol A) and cross-coupling was not favored (entries 7, 8, 9 and 10). On the other hand, appropriate feed ratio achieved hybridization in good yield and high content of poly(bisphenol A) by sufficient cross-coupling between the two polymers. Then, poly(bisphenol A) was added gradually during the oxidative coupling in order to controll the concentration of poly(bisphenol A) favorable for cross-coupling during the reaction. In this method, soluble hybrid bearing 1.61 poly(bisphenol A) unit per 1 glucose unit was synthesized. Thus, hybrid formation was much affected by the feed ratio. Appropriate reaction condition enabled sufficient hybrid synthesis and to control the composition.

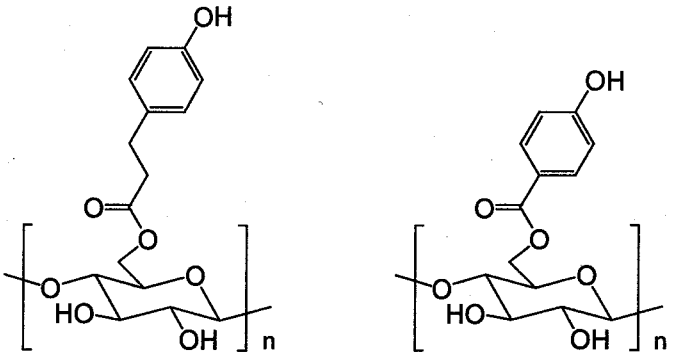
Effect of the linkage structure on the oxidative grafting of poly(bisphenol A). Effect of the linkage structure between cellulose and phenol group was investigated as shown in Figure 2. Two cellulose derivatives (**3** and **4**), which have almost the same DS as **2**, were synthesized for the oxidative coupling with poly(bisphenol A) (Chart 2). The reaction condition was subjected to that of entries 1,2 and 3 in Table 1. In the oxidative coupling in the presence of **3**, gelation took place earlier than **2**, suggesting higher reactivity of **3** for the oxidative coupling, resulting in the formation of the insoluble gel (Figure 2 (A)). In using **4** as cellulose backbone, on the other hand, the corresponding hybrid was not formed; only the homo-coupling of poly(bisphenol A) took place (Figure 2 (B)). Two factors are considered for no formation of the hybrid: shorter spacer length of **4** between the cellulose backbone and reactive phenol group, and lower oxidative reactivity of the phenol group by the substituent of electron-withdrawing carbonyl group. These data suggest that the reactivity of the phenol-containing cellulose should be controlled for the production of the soluble cellulose-phenolic polymer hybrid.

Table 1. Oxidative grafting of poly(bisphenol A) onto **2**. [a]

Entry	Poly(bisphenol A) (mg)	H_2O_2 (μmol)	Yield [b] (mg)	Unit ratio [c]
1	45.6	20	16	0.15
2	45.6	40	33	1.22
3	45.6	60	42	1.50
4	45.6	80	gel	n.d. [d]
5	0	20	gel	n.d. [d]
6	11.0	20	gel	n.d. [d]
7	91.2	40	20	n.d. [d]
8	91.2	80	21	n.d. [d]
9	91.2	120	20	n.d. [d]
10	91.2	160	17	n.d. [d]
11	34.2	12	18	0.09
12	34.2 + 3.8	24	23	0.34
13	34.2 + 3.8*2	36	35	0.92
14	34.2 + 3.8*3	48	39	1.61

[a] Grafting of poly(bisphenol A) onto **2** (17 mg) using the iron salen complex (0.66 mg) in pyridine (2 mL) at room temperature for 24 h under air.
[b] Methanol-insoluble part. [c] Unit molar ratio of bisphenol A per glucose, determined by elemental analysis. [d] Not determined.

Chart 2.



3 **4**

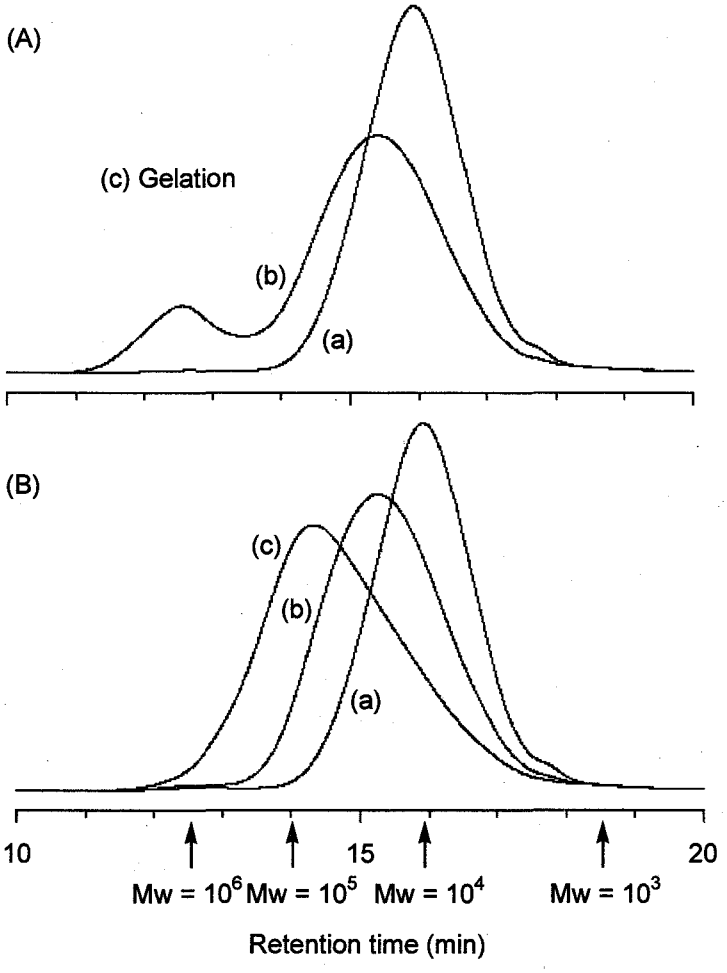


Figure 2. GPC traces of the reaction mixtures monitored by UV detector (340 nm), using (A) 3 and (B) 4. (a) Before reaction (poly(bisphenol A)), (b) H_2O_2 20 μmol, (c) H_2O_2 40 μmol.

Hydrolysis of the hybrid. In order to confirm the hybrid structure, the isolated hybrid was subjected to the alkaline hydrolysis and the hydrolyzed product was analyzed by SEC with UV detector at 340 nm. GPC traces in the case of entry 12 in Table 1 were shown in Figure 3. The hydrolyzed product showed almost the same retention time as homo-coupled poly(bisphenol A) in the reaction mixture. The SEC analysis revealed that the molecular weight of poly(bisphenol A) branch increased with the addition of hydrogen peroxide as the molecular weight of the homo-coupled poly(bisphenol A) increased similarly (Figure 4). This means that poly(bisphenol A) grafted onto the cellulose backbone at the early stage of the reaction would be further coupled with other poly(bisphenol A) molecules, leading to the increase in the molecular weight of the poly(bisphenol A) branch.

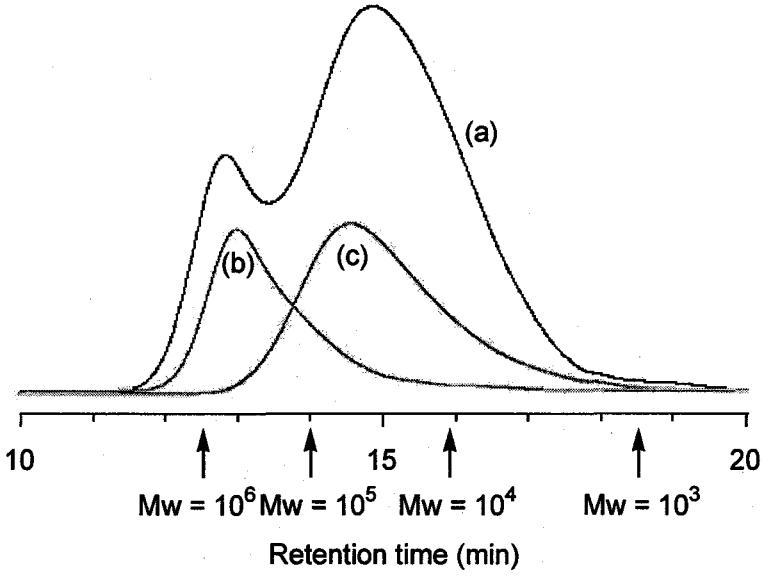


Figure 3. GPC traces of (a) reaction mixture, (b) isolated hybrid, and (c) hydrolyzed product of the isolated hybrid monitored by UV detector (340 nm).

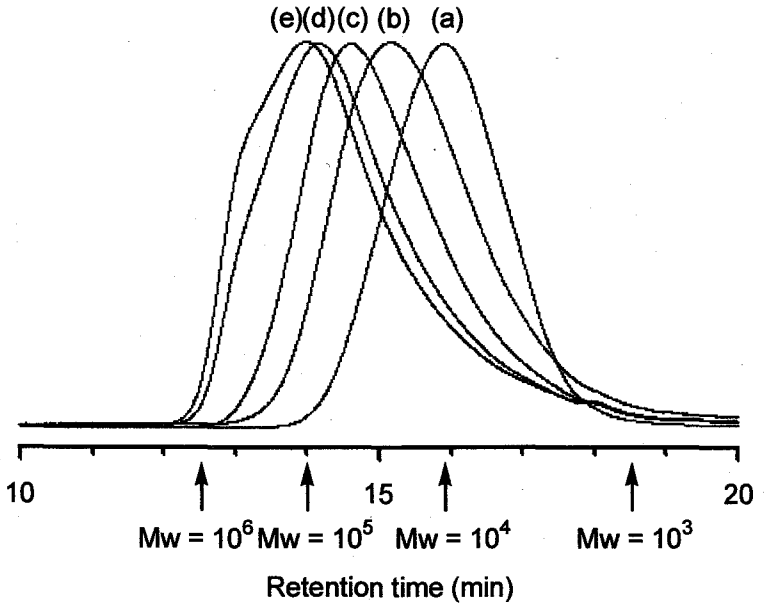


Figure 4. GPC traces of hydrolyzed products monitored by UV detector (340 nm). (a) Poly(bisphenol A), (b) H_2O_2 12 μmol, (c) H_2O_2 24 μmol, (d) H_2O_2 36 μmol, (e) H_2O_2 48 μmol.

Grafting of enzymatically polymerized phenolic polymers. Grafting of other phenolic polymers, poly(*m*-cresol)[4] and poly(*p-tert*-butylphenol),[25] on **2** has been examined. These polymers were synthesized by an enzymatic oxidative polymerization. The grafting behavior was analyzed by SEC (Figure 4). A peak was generated at the retention time of ca. 13 min as in the case with poly(bisphenol A). The outlines of the reactions were similar regardless of phenolic polymer structure although the reaction rates were considerably different. Thus different kinds of phenolic polymers could be applied to the synthesis of hybrid.

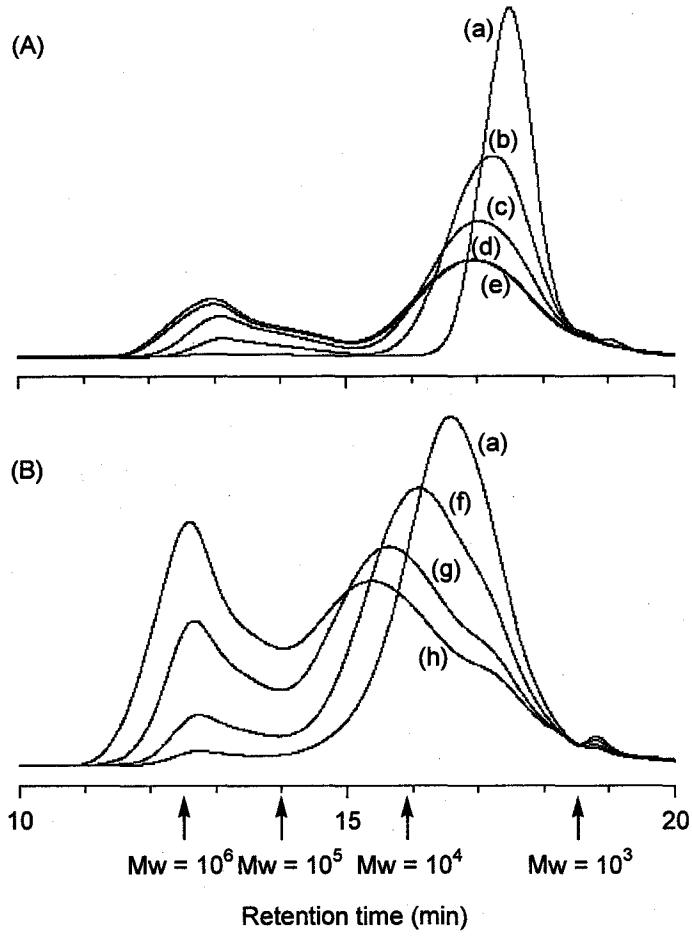


Figure 5. GPC traces of the reaction mixtures monitored by UV detector (340 nm), using (A) poly(*p-tert*-butylphenol) and (B) poly(*m*-cresol). (a) Before reaction, (b) H_2O_2 20 μmol, (c) H_2O_2 40 μmol, (d) H_2O_2 60 μmol, (e) H_2O_2 80 μmol, (f) H_2O_2 8 μmol, (g) H_2O_2 16 μmol, (h) H_2O_2 24 μmol.

Conclusion

Phenolic polymers were oxidatively grafted onto a phenol-containing cellulose backbone by using the iron salen complex as a catalyst in homogeneous system, producing cellulose-phenolic polymer hybrids, artificial wood polymers. By selecting appropriate reaction conditions, cross-coupling of both polymers preferentially took

place to produce the soluble hybrids without gelation. The hybrid composition could be controlled by the amount of hydrogen peroxide. Furthermore, the structure of phenolic polymers and of the linkage between cellulose and phenol group greatly affected the coupling behaviors. The present hybrid is expected to show good biodegradability like native wood and have high potential for various applications.

References

1) Reihmann, M. H.; Ritter, H. *Macromol. Chem. Phys.* **2000**, *201*, 798.

2) Fukuoka, T.; Tonami, H.; Maruichi, N.; Uyama, H.; Kobayashi, S.; Higashimura, H. *Macromolules* **2000**, *33*, 9152.

3) Liu, W.; Cholli, A. L.; Kumar, J.; Tripathy, S.; Samuelson, L. *Macromolecules* **2001**, *34*, 3522.

4) Tonami, H.; Uyama, H.; Kobayashi, S.; Kubota, M. *Macromol. Chem. Phys.* **1999**, *200*, 2365.

5) Uyama, H.; Lohavisavapanich, C.; Ikeda, R.; Kobayashi, S. *Macromolecules* **1998**, *31*, 554.

6) Dordick, J. S.; Marletta, M. A.; Klibanov, A. M. *Biotechnol. Bioeng.* **1987**, *30*, 31.

7) Rao, A. M.; John, V. T.; Gonzalez, R. D.; Akkara, J. A.; Kaplan, D. L. *Biotechnol. Bioeng.* **1993**, *41*, 531.

8) Uyama, H.; Kurioka, H.; Kaneko, I.; Kobayashi, S. *Chem. Lett.* **1994**, 423.

9) Hay, A. S; Blanchard, H. S.; Endres, G. F.; Eustance, J. W. *J. Am. Chem. Soc.* **1959**, *81*, 6335.

10) Hay, A. S. *J. Polym. Sci., Polym. Chem. Ed.* **1998**, *36*, 505.

11) Gross, R. A.; Kaplan, D. L.; Swift, G. Ed., *ACS Symp. Ser.* **1998**, *684*.

12) Tonami, H.; Uyama, H.; Kobayashi, S.; Higashimura, H.; Oguchi, T. *J. Macromol. Sci. - Pure Appl. Chem.* **1999**, *A36*, 719.

13) Tonami, H.; Uyama, H.; Oguchi, T.; Higashimura, H.; Kobayashi, S. *Polym. Bull.* **1999**, *42*, 125.

14) Kobayashi, S.; Uyama, H.; Kimura, S. *Chem. Rev.* **2001**, *101*, 3793.

15) Halab-Kessira, L.; Ricard, A. *Eur. Polym. J.* **1999**, *35*, 1065.

16) Mais, U.; Binder, W. H.; Knaus, S.; Gruber, H. *Macromol. Chem. Phys.* **2000**, *201*, 2115.

17) Carlmark, A.; Malmström, E. *J. Am. Chem. Soc.* **2002**, *124*, 900.

18) Shukla, S. R.; Athalye, A. R. *J. Appl. Polym. Sci.* **1993**, *48*, 1877.

19) Yoshinobu, M.; Morita, M.; Sakata, I. *J. Appl. Polym. Sci.* **1992**, *45*, 805.

20) Zhang, Z. B.; McCormick, C. L. *J. Appl. Polym. Sci.* **1997**, *66*, 307.

21) Berlin, A. A.; Kislenko, V. N. *Prog. Polym. Sci.* **1992**, *17*, 765.

22) Krässig, H. *Sven. Papperstidn.* **1971**, *74*, 417.

23) Rahn, K.; Diamantoglou, M.; Klemm, D.; Berghmans, H.; Heinze, Th., *Angew. Makromol. Chem.* **1996**, *238*, 143.

24) Pfeiffer, P.; Breith, E.; Lübbe, E.; Tumaki, T. *Ann.* **1933**, *503*, 84.

25) Mita, N.; Tawaki, S.; Uyama, H.; Kobayashi, S. *Chem. Lett.* **2002**, 402.

Concluding Remarks

This thesis describes oxidative polymerization of phenolic derivatives: preparation of new functional polyphenols, establishment of efficient reaction conditions, and development of novel reaction system. In this section, the results of investigation are briefly summarized.

In Chapter 1, HRP- and SBP-catalyzed oxidative polymerization of *m*-substituted phenols has been performed in a mixture of a water-miscible organic solvent and buffer at room temperature under air. In the polymerization of *m*-cresol using HRP catalyst, effects of an organic solvent, buffer pH, and their mixing ratio have been systematically investigated with respect to the polymer yield, solubility, and molecular weight. The difference of the polymerization behaviors, depending on the origin of peroxidases, and relationship with the *m*-substituent were examined.

In Chapter 2, HRP-catalyzed polymerization of *m*-ethynylphenol possessing two reactive groups, phenol and acetylene moieties, was carried out in an aqueous methanol under air. The reaction of the monomer using a copper/amine catalyst, a conventional catalyst for oxidative coupling, exclusively produced a diacetylene derivative. From these data, it was found that the peroxidase catalysis induced the chemoselective polymerization of the monomer. The resulting polymer was converted to carbonized polymer in a high yield and the process was analyzed by IR, Raman, and X-ray diffraction measurements.

In Chapter 3, two functional phenolic polymers were chemoenzymatically prepared. At first, 4-hydroxyphenyl benzoate was oxidatively polymerized by the peroxidase catalyst and followed by hydrolysis in alkaline solution to give poly(hydroquinone). The polymerization of tyrosine esters, followed by alkaline hydrolysis of the ester group, produced the other target, poly(tyrosine) having amino

acid moiety in the side chain.

In Chapter 4, HRP-catalyzed polymerization of *m*-substituted phenols has been achieved in the presence of heptakis(2,6-di-*O*-methyl)-β-cyclodextrin (DM-β-CD) in a buffer. A water-soluble complex of the monomer and DM-β-CD was formed and the polymerization was performed by peroxidase catalyst to give the polymer in high yields. The inclusion complex formation was examined by NMR.

In Chapter 5, oxidative polymerization of 2,6-disubstituted phenols has been performed by using an iron salen complex and hydrogen peroxide as a catalyst and an oxidizing agent, respectively. Efficient production of PPO and potential of the complex for oxidative polymerization were mentioned.

In Chapter 6, synthesis of a phenolic polymer bearing photosensitives groups was carried out and its photochemical behavior caused by UV irradiation was discussed. Spin coated polymer films were prepared and characterized. The behavior in the UV irradiation was analyzed by IR spectroscopy and waveguide spectroscopy.

In Chapter 7, oxidative grafting of phenolic polymers onto a phenol-containing cellulose has been performed in homogeneous system at room temperature under air to produce cellulose-phenolic polymer hybrids. The course of the reaction was analyzed by SEC in detail.

To conclude, the present investigation provides not only new functional phenolic polymers but also environmentally benign and efficient reaction systems to produce soluble polymers by utilizing new catalyst and method, which would be further applicable to synthesis of other functional phenolic polymers for the future. The present study is expected to develop phenolic polymer synthesis which has infinite potential in the field of science and industry.

List of Publications

Chapter 1

"Peroxidase-Catalyzed Oxidative Polymerization of *m*-Substituted Phenol Derivatives"

Tonami, H.; Uyama, H.; Kobayashi, S.; Kubota, M.

Macromol. Chem. Phys. **1999**, *200*, 2365.

Chapter 2

"Chemoselective Oxidative Polymerization of *m*-Ethynylphenol by Peroxidase Catalyst to a New Reactive Polyphenol"

Tonami, H.; Uyama, H.; Kobayashi, S.; Fujita, T.; Taguchi, Y.; Osada, K.

Biomacromolecules **2000**, *1*, 149.

Chapter 3

"Chemoenzymatic Synthesis of a Poly(hydroquinone)"

Tonami, H.; Uyama, H.; Kobayashi, S.; Rettig, K.; Ritter, H.

Macromol. Chem. Phys. **1999**, *200*, 1998.

"Enzymatic Polymerization of Tyrosine Derivatives. Peroxidase- and Protease- Catalyzed Synthesis of Poly(tyrosine)s with Different Structures"

Fukuoka, T.; Tachibana, Y.; Tonami, H.; Uyama, H.; Kobayashi, S.

Biomacromolecules **2002**, *3*, 768.

Chapter 4

"Enzymatic Polymerization of *m*-Substituted Phenols in the Presence of 2,6-Di-*O*-methyl-β-cyclodextrin in Water"

Tonami, H.; Uyama, H.; Kobayashi, S.; Reihmann, M.; Ritter, H.

e-Polymers **2002**, 3.

Chapter 5 "Oxidative Polymerization of 2,6-Disubstituted Phenols Catalyzed by Iron-Salen Complex"

Tonami, H.; Uyama, H.; Kobayashi, S.

J. Macromol. Sci.-Pure Appl. Chem. **1999**, *A36*, 719.

"Synthesis of a Soluble Polyphenol by Oxidative Polymerization of Bisphenol-A Using Iron-Salen Complex as Catalyst"

Tonami, H.; Uyama, H.; Oguchi, T.; Higashimura, H.; Kobayashi, S.

Polymer Bulletin **1999**, *42*, 125.

Chapter 6 "Synthesis of a Polyphenol with a Mesoionic 6-Oxo-1,6-dihydropyrimidin-3-ium-4-olate as Pendant Group and Its Photochemical Behavior"

Tonami, H.; Uyama, H.; Kobayashi, S.; Menges, B.; Mittler, S.;

Theis, A.; Ritter, H.

Macromol. Chem. Phys., accepted.

Chapter 7 "Oxidative Grafting of Phenolic Polymers onto Phenol-Containing Cellulose: Synthesis of a New Class of Artificial Wood Polymers"

Tonami, H.; Uyama, H.; Kobayashi, S.

Macromolecules, in preparation.

Other Publications Related to the Content of This Thesis

"Enzymatic Oxidative Polymerization of *p-t*-Butylphenol and Characterization of the Product Polymer"
Mita, N.; Maruichi, N.; Tonami, H.; Nagahata, R.; Tawaki, S.; Uyama, H.; Kobayashi, S.
Bull. Chem. Soc. Jpn. **2003**, *76*, 1.

"Peroxidase-Catalyzed Oxidative Polymerization of Bisphenols"
Uyama, H.; Maruichi, N.; Tonami, H.; Kobayashi, S.
Biomacromolecules **2002**, *3*, 187.

"Self-Association of *m*-Cresol in Aqueous Organic Solvents: Relation to Enzymatic Polymerization Reaction"
Oguchi, T.; Wakisaka, A.; Tawaki, S.; Tonami, H.; Uyama, H.; Kobayashi, S.
J. Phys. Chem. B **2002**, *106*, 1421.

"Peroxidase-Catalyzed Oxidative Polymerization of 4,4'-Dihydroxyphenyl Ether. Formation of α, ω-Hydroxyoligo(1,4-phenylene oxide) through an Unusual Reaction Pathway"
Fukuoka, T.; Tonami, H.; Maruichi, N.; Uyama, H.; Kobayashi, S.
Macromolecules **2000**, *33*, 9152.

"Peroxidase-Catalyzed Polymerization of Fluorine-containing phenols"

Ikeda, R.; Maruichi, N.; Tonami, H.; Tanaka, H.; Uyama, H.; Kobayashi, S.

J. Macromol. Sci.-Pure Appl. Chem. **2000**, *A37*, 983.

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